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In vitro characterization of bioresorbable polymers and composites for drug delivery and bone replacement

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ABSTRACT

A biodegradable device for controlled release of toremifene citrate was studied based upon ε-caprolactone and DL-lactide copolymers (P(CL/DL-LA)) and silica xerogel. The effect of copolymer composition, molecular weight of a copolymer, and drug loading on the release rate of toremifene citrate were investigated and thus it was possible to adjust the release period from 3 months to 1 year. The applicability of the P(CL/DL-LA) copolymers for matrix type controlled release devices was further studied by characterizing the interactions between copolymers and model compounds, theophylline, propranolol hydrochloride and lidocaine by differential scanning calorimetry (DSC) and molecular modelling. The hydrophilicity of the copolymers as well as the level of average molecular weight was modified using different co-initiators i.e. glycerol and polyethylene glycols. Hydrolytic degradation of the copolymers was recorded and the comparison between degradation and release profiles was obtained. The results clearly demonstrated that the desired release rates of these model compounds could be tailored by varying the compound loading, by modifying the hydrophilicity of the matrix copolymer from matrices with low lactide content and by choosing the appropriate comonomer ratio between ε-caprolactone and DL-lactide.

Two different composite materials for bone replacement were studied and their properties evaluated *in vitro*. The first material combined the P(CL/DL-LA) copolymer with bioactive glass S53P4 and the second was a composite consisting of amorphous lactic acid based poly(ester-urethane) (PEU-BDI) with hydroxyapatite (HA) or biphasic calcium phosphate (BCP). The formation of a biologically active Ca-P layer on the surfaces of the composites in hydrolysis indicates *in vitro* bioactivity and it was found to be dependent on the weight fraction and granule size range of the bioactive glass used. PEU-BDI and its composites with 20 and 40 vol.% bioceramic filler, were characterized prior to their use as biocompatible and bioabsorbable artificial bone materials. The rigidity of the materials was increased with fillers, due to good compatibility with the matrix. Processing by melt blending and sterilization by gamma-irradiation caused some chemical degradation, i.e. loss of molecular weight, but did not affect dynamic mechanical properties. The storage modulus values of all the composite materials remained within ranges that were mechanically compatible with bone over the whole five weeks of hydrolysis. The correlation of *in vitro* and *in vivo* bioactivity of the composites needs to be established, but based on the *in vitro* evaluation, the glass composites have the potential for a variety of applications as implant materials in orthopaedics and dentistry and the PEU-BDI composites have potential for application as fracture fixation materials.

PREFACE

This work was carried out at Helsinki University of Technology, Laboratory of Polymer Technology between 1995 and 2001. Part of the study was carried out during a visiting period of four months at the Interdisciplinary Research Centre (IRC) in Biomedical Materials, Queen Mary, University of London, UK. The research was started during the National Technology Agency (TEKES) "Biodegradable Polymers Technology Programme 1992-1996" and was then continued in targeted research projects. The financial support from TEKES and the Research Foundation of Helsinki University of Technology is gratefully acknowledged.

I would like to thank Professor Jukka Seppälä for his guidance and interest in my work, and for the opportunity to work in his laboratory as well as to carry out the visiting period in London. I would like to thank all my co-authors from Turku: Pirjo Kortesuo, Manja Ahola, Juha Kiesvaara, Teemu Tirri, Tiina Jaakkola, Timo Närhi, and Professor Antti Yli-Urpo. It has been both fruitful and challenging to work with researchers from different disciplines of science. Lisbeth Ahjopalo is thanked for her insight into the world of molecular modelling and she is acknowledged as a co-author. Professor Elizabeth Tanner is gratefully thanked for providing the opportunity to work at IRC and gain some insight into the workings of the interdisciplinary research institute. Whilst at IRC, I had the pleasure of working with Showan Nazhat, also acknowledged as a co-author.

My co-authors and colleagues at Helsinki University of Technology, Janne Kylmä, Jukka Tuominen, Teija Karjalainen, and Harri Korhonen, are most gratefully acknowledged for sharing the scientific work and discussions over the years as well as providing encouragement when most needed. I would also like to acknowledge the preceding work carried out by Mari Hiljanen and Petri Orava. All colleagues and personnel of the laboratory are thanked for creating such an enjoyable working environment. In particular, Jorma Hakala is thanked for his help in literature research, and Eija Ahonen and Arto Mäkinen for their technical assistance.

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Espoo, February 2002

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CONTENTS

LIST OF PUBLICATIONS

This thesis is based on the following six publications (Appendices I-VI), which are, throughout the summary, referred to by their Roman numerals.

I Ahola, M., Rich, J., Kortesuo, P., Kiesvaara, J., Seppälä, J., and Yli-Urpo, A., *In vitro* evaluation of biodegradable poly(ε-caprolactone-co-DL-lactide)/silica xerogel composites containing toremifene citrate, *Int. J. Pharm.* **181** (1999) 181-191.

II Rich, J., Kortesuo, P., Ahola, M., Yli-Urpo, Kiesvaara, J., and Seppälä, J., Effect of the molecular weight of poly(ε-caprolactone-co-DL-lactide) on toremifene citrate release from copolymer/silica xerogel composites, *Int. J. Pharm.* **212** (2001) 121-130.

III Karjalainen, T., Rich, J., and Seppälä, J., Release of model compounds from modified lactone copolymers, *J. Appl. Polym. Sci.* **81** (2001) 2118-2126.

IV Rich, J., Karjalainen, T., Ahjopalo, L., and Seppälä, J., Model compound release from DL-lactide/ε-caprolactone copolymers and evaluation of specific interactions by molecular modelling, accepted in *J. Appl. Polym. Sci.* **86** (2002) 1-9.

V Rich, J., Jaakkola, T., Tirri, T., Närhi, T., Yli-Urpo, A., and Seppälä, J., *In vitro* evaluation of poly(ε-caprolactone-co-DL-lactide)/bioactive glass composites, *Biomaterials* **23** (2002) 2143-2150.

VI Rich, J., Tuominen, J., Kylmä, J., Seppälä, J., Nazhat, S., and Tanner, K.E., Lactic acid based PEU/HA and PEU/BCP composites: dynamic mechanical characterization of hydrolysis, *J. Biomed. Mat. Res. Appl. Biomater.* **63** (2002) 346-353.

The author's contribution in the appended publications

Publications I and II: Jaana Rich has carried out the experimental work, interpretation of the results, and the preparation of the manuscripts together with Manja Ahola and Pirjo Kortesuo.

Publications III and IV: Jaana Rich and Teija Karjalainen are jointly responsible for the research plan, experimental work, interpretation of the results, and the preparation of the manuscripts. Lisbeth Ahjopalo carried out molecular modelling and interpretation of those results in Publication IV.

Publication V: Jaana Rich has participated in the preparation of the research plan and experimental work, and has written the manuscript.

Publication VI: Jaana Rich has for the main part been in charge of the research plan, experimental work, and interpretation of the results and has prepared the manuscript.

NOMENCLATURE

BCP	biphasic calcium phosphate
BDI	1,4-butane diisocyanate
Ca-P	calcium phosphates
CL	ε-caprolactone
DMA	dynamic mechanical analysis
DMTA	dynamic mechanical thermal analysis
DSC	differential scanning calorimetry
HA	hydroxyapatite
HMW	high molecular weight
LMW	lower molecular weight
PCL	poly(ε-caprolactone)
P(CL/DL-LA)	poly(ε-caprolactone/DL-lactide)
P(CL80/LA20)	poly(ε-caprolactone/DL-lactide), comonomer ratio in weight per cents
PEG	poly(ethylene glycol)
PEU-BDI	poly(ester-urethane) linked with 1,4-butanediisocyanate
P(DL-LA)	poly(DL-lactide)
PLA	poly(lactide)
P(L-LA)	poly(L-lactide)
SBF	simulated body fluid
SEC	size exclusion chromatography
SEM	scanning electron microscopy
TCP	tricalciumphosphate

SYMBOLS

E'	storage modulus (Pa)
E''	loss modulus (Pa)
ΔE_{tot}	energy of mixing (kcal mol^{-1})
E_{AA}, E_{BB}, E_{AB}	interaction energies between chain segments A and B (kcal mol^{-1})
$\overline{M}_n$	number average molecular weight (g mol^{-1})
$\overline{M}_w$	weight average molecular weight (g mol^{-1})
MWD	molecular weight distribution
T_g	glass transition temperature (°C)
T_m	melting temperature (°C)

1 INTRODUCTION

1.1 General background

In the last few decades interest in biodegradable polymer materials has been steadily increasing and these polymers currently have two major application areas; biomedical polymers and mass-produced applications such as packaging (Amass *et al.* 1998, Ikada and Tsuji, 2000). Bioresorbable polymers degrade in the physiological environment and the by-products are eliminated or completely bioabsorbed. In comparison, according to the strict definition, biodegradable polymers require enzymes of microorganisms for hydrolytic or oxidative degradation. Generally, a polymer that loses its weight over time in the living body is called an absorbable, resorbable, or bioabsorbable, as well as a biodegradable polymer. This terminology applies regardless of its degradation mode, in other words for both enzymatic and non-enzymatic hydrolysis. Biodegradable polymers can be classified on the basis of their origin, either naturally occurring or synthetic. Among synthetic resorbable polymers for implants, polyhydroxyacids occupy the main position. These are mainly poly(L-lactide), poly(glycolide) and copolymers based on L-lactide, L/DL-lactide, DL-lactide, glycolide, trimethyl carbonate and ε-caprolactone (Gogolewski, 2000). The largest and most enduring use of biodegradable polymers is for suturing (Ikada and Tsuji, 2000). Potentially, devices made from bioresorbable polymers can overcome problems associated with metal implants, such as stress protection, potential for corrosion, wear and debris formation, as well as the necessity of implant removal (Gogolewski, 2000). Resorbable polymers have proven to be good materials for a range of devices in trauma surgery.

Many types of surgically implantable devices that only function for a relatively short time *in vivo* can be made from biodegradable polymers. These devices are eliminated from the body by hydrolytic degradation and subsequent metabolism after serving their intended purpose. The general criteria for selecting a polymer for use as a surgical biomaterial is to match the mechanical properties and time of degradation to the needs of the application. A further advantage of bioabsorbable polymers over nondegradable materials relates to the control over the rate at which mechanical properties deteriorate *in vivo*. Thus, fracture fixation plates, which gradually lose stiffness, are postulated to provide a superior result compared to steel plates, which ultimately weaken bone due to constant stress shielding (Barrows, 1991). Bioabsorbable devices represent the state of the art in managing orthopaedic problems and the use of implants in fracture fixation, drug delivery or ligament repair will become even more common in the next century of orthopaedic care (An *et al.*, 2000). Biodegradable polymers in drug delivery technology require no follow-up surgical removal once the drug supply has been depleted. They have mainly been studied in injectable microsphere formulations, although implantable rods and pellets are also being investigated (Lewis, 1990). Resorbable materials are particularly promising as short-term or transient implants, namely bone plates, screws, pins, rods, ligaments, tendons, bone replacement, vascular grafts, and artificial skin (Ramakrishna *et al.*, 2001).

1.2 Biomaterials and prerequisites for biomedical applications

A biomaterial is a non-viable material used in a medical device that is intended to interact with biological systems (Williams, 1990). The biomaterials spectrum includes metals and alloys, polymers, plastics, elastomers and fibres, ceramics and glasses, composites and natural materials and their synthetic analogues. Due to the close contact of biomaterials with biological systems, the interaction and biocompatibility of synthetic polymers are of prime concern. The properties required of polymeric biomaterials are similar to other biomaterials, i.e. biocompatibility, sterilizability, adequate mechanical and physical properties, and manufacturability. Biocompatibility means the acceptance of an implant by surrounding tissues and by the body as a whole. The implant should be compatible with tissues in terms of mechanical, chemical, surface, and pharmacological properties. Biocompatibility is defined as the ability of a material to perform with an appropriate host response in a specific application (Williams, 1987), where host response is the reaction of a living system to the presence of a material. Body fluids can act as a solvent for impurities entrapped within a polymer matrix. On the other hand, compounds issued from polymerisation and processing stages, namely monomers, oligomers, initiators, solvents, etc., can generate particular morphological characteristics such as crystallinity or porosity, and cause toxicity or undesired physical aging due to slow release or slow uptake of low molecular weight compounds according to phase partition (Vert, 2000).

Ceramics, glasses and glass-ceramics used in biological applications are referred to as bioceramics. They can be divided into relatively inert, bioactive or surface reactive, and biodegradable or resorbable types (Hench and Wilson, 1993). Bioactive materials can bond to bone chemically and are defined as materials that elicit a specific biological response at the interface of the materials resulting in the formation of a bond between the tissues and material (Hench and Andersson, 1993). Since the discovery of Bioglass® by Hench and co-workers (1971) the bioactivity of a variety of glass compositions has been well established (Kokubo *et al.*, 1990a, Andersson *et al.*, 1990, Brink *et al.*, 1997). Resorbable ceramics degrade on implantation in the host and the rate of degradation varies from material to material (Bajpai and Billotte, 1995). Almost all bioresorbable ceramics are variations of calcium phosphate. Several types of calcium phosphate (Ca-P) materials are used in bone repair and augmentation (LeGeros and LeGeros, 1996) and it is known that Ca/P ratios within the range 1.50 to 1.67 promote bone ingrowth (Klein *et al.*, 1983). Because most structural living tissues (bone, ligament, connective tissue etc.) are macromolecular composites, synthetic polymeric composites are an attractive group of materials for the development of new, tailor-made biomaterials for replacing, supporting, augmentation or fixation of living tissues (Törmälä and Pohjonen, 1997). The original concept of bioceramic reinforced polymer composites, i.e., ceramic materials conferring stiffness and biological activity to the composite material while the viscoelastic polymer compensates for the brittle ceramics, was introduced by Bonfield and co-workers in early 1980´s (Bonfield *et al.*, 1981). The properties of the composite material depend on the shape and size of the filler, the volume fraction and the integrity of the interface between the constituents.

The two major routes in obtaining an acceptably sterile product are aseptic manufacturing and terminal sterilisation. For economical and practical reasons, the latter strategy is considered a more realistic approach to achieve sterile biomedical devices. Commonly used sterilisation techniques are dry heat, autoclaving, ethylene oxide gas, and radiation (Lee *et al.*, 1995). Low-temperature radio-frequency glow discharge plasma treatment has also been introduced as a sterilization method for polyester devices (Holy *et al.*, 2001). Conventional methods such as dry or moist heat sterilization often initiate degradation and hydrolysis of the devices used, and ethylene oxide, due to residual amounts, often causes toxicological problems. Thus, irradiation sterilization of pharmaceutical preparations has become popular in recent years. Radiation sterilization causes alterations in the molecular structures of the polymers, which appear as changes in the chemical or physical properties (Sintzel *et al.*, 1997). Sterilizability should thus be considered early in the development of new biomaterial devices.

1.3 Synthetic bioresorbable polyesters

Biodegradation can be accomplished by synthesizing polymers that have hydrolytically unstable linkages in the backbone. The most common chemical functional groups of this kind are esters, anhydrides, orthoesters, and amides. Depending on the chemical structure of the polymer backbone, degradation can occur by either surface or bulk erosion. Surface erosion occurs when the rate of erosion exceeds the rate of water penetration into the bulk of the polymer. This type of degradation can be obtained in poly(anhydrides) and poly(ortho esters) (Domb *et al.*, 1997, Heller *et al.*, 2000). The hydrolysis of bulk degrading bioresorbable polymers usually proceeds by loss of molecular weight at first, followed by loss of mass in the second stage. Generally, hydrolysis (including enzyme-mediated hydrolysis) is the most probable degradation mechanism for hetero-chain polymers *in vivo* (Williams, 1994). The degradation of poly(ε-caprolactone) (PCL) and related polyesters such as poly(lactide) (PLA) and its copolymers first involves non-enzymatic hydrolysis of ester linkages, autocatalyzed by the generation of carboxylic acid end groups, followed by the loss of mass (Ali *et al.*, 1993). The role of enzymes in the degradability of aliphatic polyesters is under investigation (MacDonald, 1996, Gan *et al.*, 1999, Chen *et al.*, 2000, Li *et al.*, 2001).

The factors that affect the mechanical performance of biodegradable polymers are monomer selection, polymerisation and process conditions, and the presence of additives (e.g. fillers). These factors, in turn, influence the hydrophilicity, crystallinity, melt and glass transition temperatures, molecular weight, molecular weight distribution, end groups, sequence distribution (random versus block), and the presence of residual monomer or additives in the polymer (Middleton and Tipton, 2000). Furthermore, all these together then influence the rate of biodegradation of the polymer.

Aliphatic polyesters can be synthesized by polycondensation from a mixture of a diol and

diacid, from a hydroxy acid or by ring-opening polymerisation of lactones. Polycondensation requires high temperatures and long reaction times to produce high molecular weight chains. In contrast ring-opening polymerisation can be used to prepare high molecular weight polymers in short periods of time under relatively mild conditions (Löfgren *et al.*, 1995). Ring-opening polymerisation of lactones can be initiated by different mechanisms. When stannous octoate is used as a catalyst, it first reacts with compounds containing hydroxyl groups forming tin alkoxide, which then acts as an actual initiator in the polymerisation (Duda *et al.*, 2000). The structure of the polymer depends on these alcohols used as co-initiators. Mono- and difunctional alcohols yield linear polymers, while alcohols with hydroxyl functionality higher than two give star- or comb-shaped polymers (Schindler *et al.*, 1982, Korhonen *et al.*, 2001). Another effective way of producing high molecular weight polyesters is to treat condensation polymers of lactic acid with chain extenders. Among chain linking agents, the extremely high reactivity of the diisocyanates has encouraged their use for coupling or chain extension of oligomers (Hiltunen *et al.*, 1997).

Lactide is the cyclic dimer of lactic acid, which exists in three stereoisomeric forms, L-lactide, naturally occurring isomer, D-lactide and meso-lactide, which contains a L-lactyl unit and a D-lactyl unit in the ring. Additionally, DL-lactide is an equimolar mixture of L- and D-lactides. Poly(L-lactide) (P(L-LA)) exhibits high tensile strength and low elongation and consequently has a high modulus that makes it more applicable than the amorphous polymers for load-bearing applications such as orthopaedic fixation and sutures. P(L-LA) has a melting point around 170°C and glass transition temperature in the range of 55-60°C. Poly(DL-lactide) (P(DL-LA)) is an amorphous polymer (T_g 45-55°C), having a random distribution of both isomeric forms of lactic acid and lacking the ability to arrange into a crystalline organized structure. P(DL-LA) has a lower tensile strength, slightly higher elongation and substantially more rapid degradation time, making it more attractive for use in drug delivery system. PCL is a ductile semicrystalline polymer, melting in the range of 54-64°C. The glass transition temperature of –60°C can be increased by copolymerisation with lactide, which also enhances the biodegradation of the polymer. PCL has good permeability to many therapeutic drugs and has been studied for long-term contraceptive delivery (Pitt, 1990).

The *in vivo* elimination time of bioresorbable polymers is determined by the nature of the polymer chemical linkage, the solubility of the degradation products, the size, shape and density of the device, the drug and additive content, the molecular weight of the polymer, and the implantation site (Domb *et al.*, 1998). Semicrystalline P(L-LA) has a degradation time in the order of 3 to 5 years, whereas P(DL-LA) degrades in 12 to 16 months (Pitt *et al.*, 1981, Bergsma *et al.*, 1995). Polyhydroxyacids degrade to monomeric acids and subsequently to carbon dioxide and water. These are removed from the body via respiratory routes and the kidneys (the Krebs cycle) (Grijpma *et al.*, 1991, Gogolewski, 2000). Copolymers of ε-caprolactone with L- or DL-lactide have been reported to induce only mild foreign-body reactions (den Dunnen *et al.*, 1997, Tomihata *et al.*, 1998), and aliphatic polyesters in general are considered biocompatible (Vert *et al.*, 1992).

1.4 Biomedical applications

Controlled release delivery systems

Controlled release dosage forms enhance the safety, efficacy and reliability of drug therapy, particularly for drug substances with a narrow therapeutic index (Thombre and Cardinal, 1988). They regulate the drug release rate to control the drug action, and reduce the frequency of drug administration to encourage the patients to comply with dosing instructions. Conventional dosage forms often lead to wide swings in serum-drug concentrations (Figure 1). Most of the drug content is released soon after administration, causing drug levels in the body to rise rapidly, peak, and then decline sharply. Since each drug has a therapeutic range above which it is toxic and below which it is ineffective, oscillating drug levels may cause alternating periods of ineffectiveness and toxicity (Edgren *et al.*, 1993, Langer, 1995).

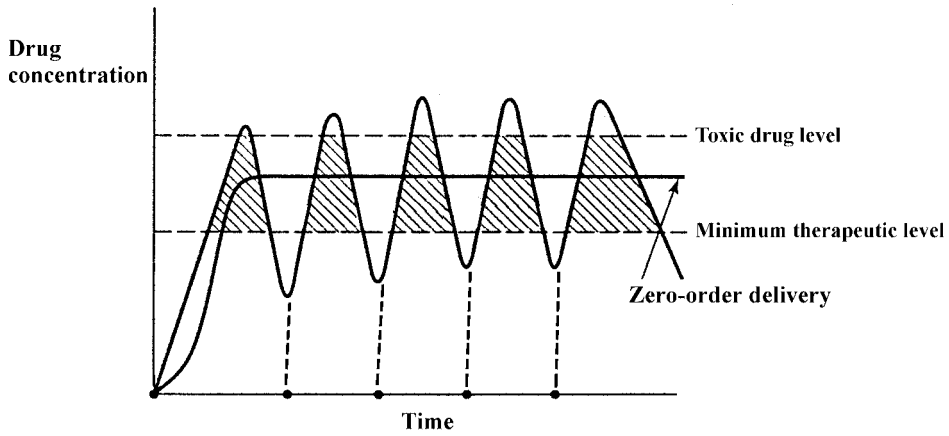


Figure 1. Controlled delivery versus immediate release delivery with repeated administration.

Controlled delivery devices are generally diffusion-based release systems applicable to the release of drugs intended for the systemic circulation or for a localized site (Griffith, 2000). Diffusion is defined as a process of mass transfer of individual molecules of a substance, brought about by random molecular motion and associated with a concentration gradient (Martin, 1993). In monolithic devices, the drug is uniformly mixed with the polymeric matrix and is present either in dissolved or dispersed form (Baker, 1987). Release follows Fickian kinetics from devices where the drug is dissolved. When the drug is dispersed in the matrix, it is released according to square root of time kinetics until the concentration in the matrix falls below the saturation value. The range of formulation variables available to control the rate of drug release from controlled-release devices is broad. The desired drug release profile in bulk degrading systems can be obtained by adjusting the molecular weight of the polymer, comonomer composition, polymer crystallinity, shape and preparation method of the device, interaction between polymer and drug, and drug loading (Buntner *et al.*, 1996, Vandamme and Mukendi, 1996, Ye and Chien, 1996, Miyajima *et al.*, 1997 & 1998, Lemmouchi *et al.*, 1998).

Bioresorbable composite materials for tissue engineering

Tissue engineering can be defined as the application of scientific principles to the design, construction, modification, growth, and maintenance of living tissues. Tissue engineering can be divided into two broad categories: 1) *in vitro* construction of bioartificial tissues from cells isolated by enzymatic dissociation of donor tissue and 2) *in vivo* alteration of cell growth and function. The first category of applications includes artificial tissues (i.e., tissues that are composed of natural and synthetic substances) to be used as an alternative to organ transplantation. For tissue engineering *in vivo*, the objective is to alter the growth and function of cells *in situ*, an example being the use of implanted polymeric tubes to promote the growth and reconnection of damaged nerves (Berthiaume and Yarmush, 1995). Polymeric biomaterials in tissue engineering research are being applied in conducting, guiding, and inducing tissue formation as well as in blocking tissue interactions. These polymers for structural scaffolds require suitable physical and mechanical properties, designable biodegradability, non-toxicity, good biocompatibility, and the ability to interact with specific cells. Scaffolds meeting these requirements may be useful in regenerating damaged tissues or organs by combining scaffolds with living cells (Han *et al.* 1998).

Figure 2 shows the integration of material and engineering properties in order to achieve a successful biomaterial for tissue regeneration. In addition, there are the biological and medical requirements that need to be satisfied in the appropriate design of a new material (Seal *et al.*, 2001).

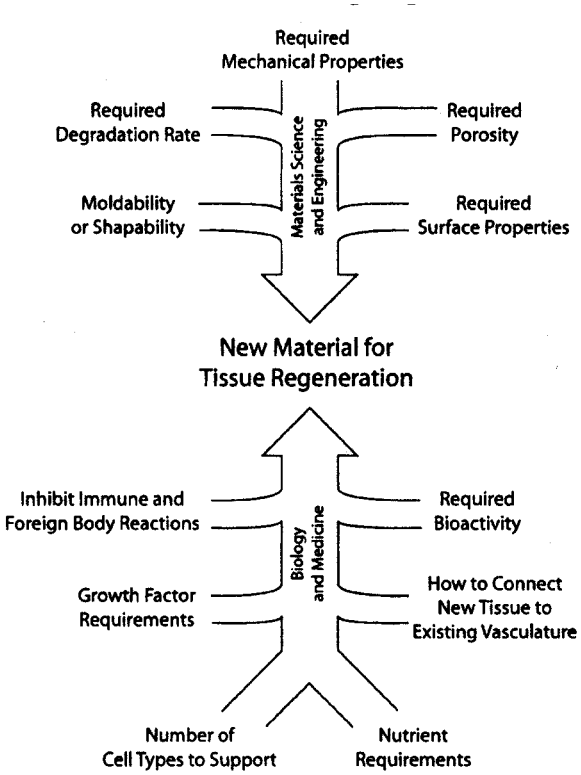


Figure 2. Illustration of how some material, biological, medical and engineering properties must be integrated to achieve successful biomaterials for tissue regeneration (Seal *et al.*, 2001).

The idea of using polymers as binders for particulate bioceramics to produce composites with improved handling and retention characteristics and to overcome the problem of brittleness associated with ceramic bone repair implants has been evaluated as an attractive approach. The critical properties of a composite implant material to be used in load-bearing areas include the degree of strength retention over time, and the structural and mechanical equivalence to bone. However, the main requirements for a bone-filling material are its degradation rate, it should degrade at about the same rate as the new hard tissue is formed, and also its handling properties, which means that it should be easy to place tightly within the gap in the bone (Ural *et al.*, 2000).

1.5 Scope of the study

The polymerisation of lactic acid based biodegradable polymers and copolymers of lactic acid or lactide with ε-caprolactone has been extensively studied over the last decade in the laboratory at Helsinki University of Technology (Hiltunen, 1997, Kylmä, 2001, Hiljanen, 2001). Bioresorbable polymers have been synthesized by ring-opening polymerisation or polycondensation followed by chain linking. As the synthesis of these polymers, characterization of mechanical properties and hydrolytic degradation was under way, it also became of interest to study the application areas of these bioresorbable polymers, especially in biomedical field.

This thesis discusses the research reported in six appended publications (I-VI). In the first four publications (I-IV) copolymers of DL-lactide and ε-caprolactone were modified and characterized *in vitro* for controlled drug delivery applications. Bioactive composite materials for bone replacement were characterized in Publications V and VI. Figure 3 shows the structure of poly(ε-caprolactone/DL-lactide) P(CL/DL-LA) used in Publications I-V and Figure 4 shows the structure of lactic acid based poly(ester-urethane) (PEU-BDI) used in study VI.

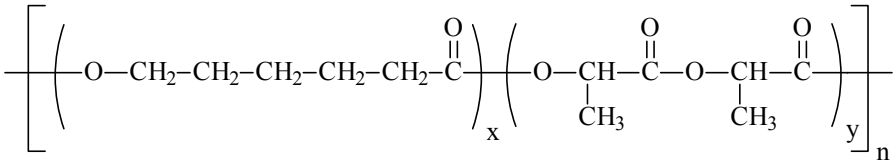
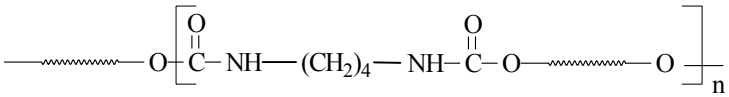


Figure 3. Structure of the bioresorbable copolymer (P(CL/DL-LA).

Biodegradable devices for the controlled release of toremifene citrate were developed in Publications I and II. Toremifene citrate is an antiestrogenic compound that exerts its antitumor action through inhibition of the estrogen-mediated growth stimulus (Valavaara, 1990). Antiestrogens have been used in the systemic treatment of hormone-dependent breast cancer (Kallio *et al.*, 1986) and thus local hormone therapy after breast cancer surgery could provide targeted and long-lasting disease control. The effects of comonomer ratio, average molecular weight, drug load, and incorporation of toremifene citrate into silica xerogel on the release were studied. The release properties of P(CL/DL-LA) copolymers were further characterized in

Publications III and IV. The release of two model compounds, theophylline and propranolol hydrochloride, at different loadings, was carried out as the hydrophilicity of the copolymers was modified using different co-initiators in Publication III. Release properties of the copolymer matrices with low caprolactone content were characterized in Publication IV, where molecular modelling was additionally applied to provide insight into the interactions between the polymer matrix and the active component.



Poly(ester-urethane), PEU-BDI

where ~~~ is lactic acid prepolymer (Mw ~30 000 g/mol)

Figure 4. Structure of lactic acid based poly(ester-urethane).

Ductile P(CL/DL-LA) copolymer with minor lactide content with bioactive glass for the filling of bone defects and guided tissue regeneration was studied in Publication V. Different amounts of bioactive glass were incorporated in the matrix in order to obtain slower (40 wt.% glass) or accelerated bioactivity (60 or 70 wt.%), i.e., slower or faster formation of a silica gel layer on the surface of the composite in dissolution. Two ranges of granule size were chosen for the experiment in order to change the area/volume ratio of the bioactive glass in the composite. The molecular weight and the melting temperature of the copolymer matrix were adjusted to enable the application of the composite material by injection below 50°C. The preparation, morphology, dynamic mechanical properties, and *in vitro* hydrolysis of the composite material were reported.

Highly crystalline polymers, such as P(L-LA), can disintegrate into crystalline particles, which can cause late foreign body reactions (Bergsma *et al.*, 1995). A solution to this is to use amorphous polymers, which degrade without generation of crystalline remnants. Polyurethanes, on the other hand, have been used for a variety of biomedical applications due to their excellent physical properties and relatively good blood compatibility, and because they can be readily tailored either by changing the components used or by changing the length of prepolymer. In this study, lactic acid prepolymer was linked with 1,4-butane diisocyanate (BDI) and, due to the relatively long poly(lactic acid) prepolymer chains, the amount of urethane units in PEU-BDI was below 2 wt.%. Therefore, the physical properties of PEU-BDI are similar to amorphous P(DL-LA), but additional improvements in compatibility and adhesion at the interface of fillers and matrix have been obtained in association with the urethane groups (Hiljanen-Vainio *et al.*, 1997, Kylmä *et al.* 2000 & 2001b). A composite material consisting of amorphous PEU-BDI reinforced with bioceramic fillers was developed and characterized *in vitro* for use as a bone replacement material in Publication VI.

2 EXPERIMENTAL

2.1 Materials

ε-Caprolactone (Fluka or Solvay Interox Ltd.) was dried over molecular sieves. DL-lactide (Purac) was recrystallized from toluene. Purified lactide was dried at 40°C for 24 h under reduced pressure before polymerisation. L-Lactic acid (88% L-lactic acid in water, 99% optically pure; ADM: Archer Daniels Midland Co.) was purified by distillation under vacuum. Sn(II) octoate (Sigma Aldrich Chemical Co.), glycerol (Rhône-Poulenc or Prolabo), 1,4-butanediol (Acros Organics) and 1,4-Butane diisocyanate (Aldrich) were used as received. Polyethylene glycol (PEG) 1000 and 4000 (Fluka) were dried under reduced pressure for 24 hours before polymerisations. Theophylline (Fluka), propranolol hydrochloride (Fluka), lidocaine (Sigma-Aldrich Chemie Gmbh), 0.85w/v saline (Sigma Diagnostics) and buffer solutions pH 7.0 (Reagecon) were used as received. Chemical structures of the drugs used as model compounds are shown in Figure 5 and some of their characteristics are listed in Table 1 (Publications III and IV).

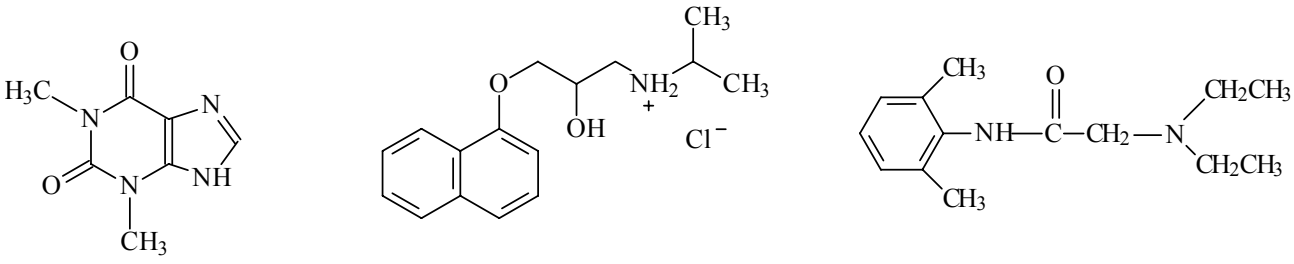


Figure 5. Structure of the used model compounds.

Table 1. Characteristics of the model compounds used in Publications III and IV.

Model compound	*Molecular weight (g mol^{-1})*	*Melting temperature (°C)*	*Maximum absorption wavelength (nm)*
Theophylline	180	274-275	275
Propranolol hydrochloride	259	163-164	214
Lidocaine	234	66-69	262

Bioactive glass S53P4 (Abmin Technologies) consisted of 53 wt.% SiO_2, 23 wt.% Na_2O, 20 wt.% CaO, and 4 wt.% P_2O_5 (Publication V). The granule size ranges were < 45 μm and 90 – 315 μm. The density of the bioactive glass was 2.66 g cm^{-3}. Two bioceramic fillers were used, synthetic hydroxyapatite (HA) (P218 grade, Plasma Biotal Ltd) and biphasic calcium phosphate (BCP) prepared from Merck calcium phosphate ("tricalciumphosphate", Merck GmbH) (Publication VI). Tricalciumphosphate (TCP) was calcinated at 900°C for 2 hours, which

transformed it to a crystalline biphasic calcium phosphate containing approximately 70% β-TCP and 30% HA, the Ca/P ratio was 1.56 (Bleach *et al.*, 2002). The mean particle diameters of the fillers were $d_{0.5}$= 2.99 μm for HA and $d_{0.5}$= 3.44 μm for BCP. The specific surface areas were substantially different at 16.3 and 3.15 m^2 g^{-1} respectively; the densities of the fillers were 3.16 g cm^{-3} (HA) and 3.12 g cm^{-3} (BCP).

The silica xerogel used was prepared using the sol gel method described in detail in Publications I and II. Toremifene citrate (Orion Corporation) was used as a model drug, which was either adsorbed on the silica surface (Publication I) or impregnated during the sol-gel process (Publication II).

2.2 Polymerisation procedures

The ring-opening polymerisation of ε-caprolactone and DL-lactide was carried out in bulk under an argon atmosphere with Sn(II) octoate as an initiator. Glycerol was used as a co-initiator in most polymerisations (0.05-0.5 mol%) but additionally polyethylene glycols were used in Publication III as co-initiators (0.1 and 0.5 mol%). Typically (Publications I and II), a batch of 300 grams of monomers, initiator and co-initiator in a glass polymerisation flask with magnetic stirrer was immersed in an oil bath. The polymerisation time was 54 hours at 120°C or 24 hours at 140°C. The polymerisation of ε-caprolactone and DL-lactide in Publications III, IV and V were also carried out in bulk. Typically, 1000 grams of monomers, Sn(II) octoate as an initiator and co-initiator were charged into a 2.5-litre batch reactor (Design Integrated Technologies) designed for the agitation of viscous materials. The polymerisations were carried out at 160°C for 4.5 to 8 hours under a nitrogen atmosphere. The copolymers were stored in dry conditions and used without further purification.

The hydroxyl-terminated prepolymer for poly(ester-urethane) synthesis was condensation polymerised in a rotation evaporator using 1,4-butanediol and Sn(II) octoate as a catalyst to produce hydroxyl-terminated oligomer. The chain linking polymerisation of the prepolymer was then carried out in a batch mixer (Brabender W50EH) with the use of 1,4-butane diisocyanate (BDI) as a chain extender. The synthesis of poly(ester-urethane) is described in detail in Publication VI. The polymer was purified by precipitating it out from a dichloromethane solution with ethanol. Composite and PEU-BDI samples in study VI were sterilized by γ-irradiation, with a nominal dose of 2.9 Mrad (Isotron plc).

2.3 Preparation of specimens

P(CL/DL-LA) copolymers were blended with toremifene citrate, silica xerogel, or bioactive glass in a batch mixer (Brabender W50EH) at 100°C for 5 minutes at 60-75 rpm (I, II, V). HA and BCP

fillers were blended with PEU-BDI in a Brabender mixer at 130°C for 5 min at 60 rpm (VI). Model compounds (theophylline, lidocaine, and propranolol hydrochloride) were mixed into P(CL/DL-LA) in a co-rotating twin-screw midi extruder (DMS, capacity 16 cm^3, screw length 150 mm) (III and IV). In the case of blend devices, the model compound was mixed together with the blend components in one batch. The midi extruder had a back-flow channel and was operated batch-wise. The screw speed was 75 rpm and the mixing time was three minutes at 100°C.

Test specimens were prepared either by compression moulding or injection-moulding. The disc specimens (diameter 10 mm, thickness 2 mm) were compression moulded (Fontijne TP400) (II and V) and square shaped test specimens (10x10x3 mm^3 (I) or 10x10x0.6 mm^3 (II)) were prepared by punching them out from compression-moulded plates with an Elastocon EP 02 puncher. Rectangular shaped devices (10x4 mm^2, thickness 1.8 mm) and specimens for dynamic mechanical thermal analysis (DMTA) measurement were prepared using a mini injection-moulding machine attached to the midi extruder (DMS) (III, IV and VI).

2.4 Hydrolysis tests

Hydrolysis tests were carried out in buffer solution (pH 7.0 at 20°C) (III, IV), simulated body fluid (SBF K9, pH 7.25 at 37°C, Kokubo *et al.*, 1990b) (I, II, V), or 0.85w/v saline solution (VI). In all studies, a number of test specimens were immersed individually in the dissolution medium (10-25 mL) and in release tests the medium was changed to maintain sink conditions. The test tubes were placed in an incubator or mixed air bath maintained at 34°C (I and II) or 37°C (III-VI). The specimens were recovered at predetermined times and weighed. Specimens were then vacuum-dried for six days at room temperature, weighed again and stored in a desiccator for further characterization; size exclusion chromatography (SEC), differential scanning calorimeter (DSC) analysis or dynamic mechanical thermal analysis (DMTA) (IV-VI). The amount of released model compound (I-IV) was determined from the solution medium by an UV-visible spectrophotometer (Hewlett Packard 845/A or Unicam UV/VIS spectrometer) at the maximum absorbance of toremifene citrate (278 nm), theophylline (275 nm), propranolol hydrochloride (214 nm) and lidocaine (262 nm). Degradation of the silica xerogel was determined by measuring dissolved $Si(OH)_4$ spectrophotometrically as molybdenum blue complex at 820 nm (I and II).

2.5 Characterization

Molecular weights were determined by room temperature SEC (Waters System Interface module, Waters 510 HPLC Pump, Waters 410 Differential Refractometer, Waters 700 Satellite Wisp, and four linear PL gel columns: 10^4 Å, 10^5 Å, 10^3 Å and 100 Å connected in series). Chloroform was used as solvent and eluent for polymers. The samples were filtered through a 0.5 μm Millex SR

filter. The injected volume was 200 μL and the flow rate was 1 mL min^{-1}. Monodisperse polystyrene standards were used for primary calibration.

The structures of the copolymers in Publications I and II were determined with a Varian Unity 400 NMR spectrometer working at 100.577 MHz for ^{13}C and at 399.96 MHz for ^{1}H. The measurement temperatures were 45°C for ^{13}C NMR and 18°C for ^{1}H NMR. Tetramethylsilane was used as an internal standard. The structures of copolymers in Publications III-V were determined with a Varian Gemini 2000, 300 MHz NMR spectrometer working at 75.452 MHz for ^{13}C and at 300.032 MHz for ^{1}H. The measurements were carried out at room temperature.

Thermal properties were measured by DSC and DMTA. The DSC measurements (Polymer Laboratories or Mettler) were carried out in the temperature range between -100°C and +200°C depending on the polymer and the heating rate was 10°C min^{-1} or 40°C min^{-1} (I-VI). Nitrogen was used as a sweeping gas. The samples (6-8 mg) were heated twice to ensure that their thermal histories were similar. Changes in crystallinity of the devices as a function of hydrolysis time were evaluated from the first heating scan in Publications II and III. The DMTA measurements were performed on a Perkin Elmer 7 Series Thermal Analysis System instrument (IV-VI). The measurements were carried out using the three-point bending over a temperature range of -90 to 100°C (IV), -90 to 45°C (V) or 20 to 80°C (VI) at a heating rate of 4°C min^{-1} and a frequency of 1 Hz.

The morphology of the cryogenically fractured samples was examined by scanning electron microscopy (SEM) (JEOL JSM-840A, JSM-6335F or Zeiss Digital Scanning Microscope 962). Surfaces were coated with a thin layer (10-20 nm) of platinum or gold before examination. (I, IV, V and VI)

2.6 Molecular modelling

For molecular modelling, the Polymer software package by Molecular Simulations Inc. was used. The calculations were carried out on a Silicon Graphics workstation Indigo^2 Impact 10000. The structure of theophylline was optimised using the semiempirical AM1 method. The partial charge distribution (ESP charges) and the dipole moment were also calculated. ESP charges are the partial charges of the atoms in the molecule; optimised to reproduce the quantum chemically calculated electrostatic potential around the molecule. The molecular mechanics and dynamics simulations were performed using the Discover 97.0/4.0.0.P software by MSI16. The polymer consistent force field (pcff) was used in these calculations.

3 RESULTS AND DISCUSSION

3.1 Copolymer/silica xerogel composites for controlled release of toremifene citrate

The copolymers exhibit a broad range of properties depending on the type and proportions of their constituent monomers. PLA, PCL, and copolymers of lactide (LA) and ε-caprolactone (CL) have been studied as controllable dosage forms that biodegrade after drug exhaustion (Pitt *et al.*, 1979, Lemmouchi and Schacht, 1997). Different comonomer ratios of the copolymers are designated here with the weight ratios of comonomers in the feed, e.g. P(CL80/LA20) consists of 80 wt.% of ε-caprolactone and 20 wt.% DL-lactide. The goal of the study was to develop a controlled release formulation of toremifene citrate using biodegradable delivery systems based upon ε-caprolactone and DL-lactide copolymers and silica xerogel (Publications I and II). The effect of copolymer composition, molecular weight of a copolymer, and drug loading on the release rate of toremifene citrate were investigated. In addition, the role of hydrophilic silica xerogel in the release of the drug from different copolymers was examined. Toremifene citrate was either adsorbed on the silica surface (Publication I) or impregnated during the sol-gel process (Publication II). The devices studied were of an appropriate size for subcutaneous or intramuscular implantation, i.e. discs.

3.1.1 Effect of copolymer composition and drug load

P(CL/DL-LA) copolymers with four different comonomer ratios were polymerised and both copolymer matrix and copolymer/silica xerogel composite devices containing the drug were prepared. The copolymer devices contained 2 wt.% of the drug and composite devices contained 20 wt.% silica xerogel, corresponding to 1.6 wt.% drug content. The results from copolymer matrices and silica xerogel composite devices for different comonomer ratios are shown in Figure 6. The total release time of toremifene citrate increased from about 70 to 200 days with increasing weight fraction of ε-caprolactone in both types of devices. Copolymers containing larger amounts of DL-lactide (40-80 wt.%) were not suitable matrices to deliver toremifene citrate in a controlled manner because of the burst effect observed at about 80 days. At that time, the rate of release changed significantly and the desired steady release rates over the whole release period were not obtained. The onset of rapid drug release may be attributed to the hydrolytic degradation of the matrices. The degradation effects were not studied here but were later confirmed to be a significant factor in determining the release properties of high lactide content copolymers (Publication IV.)

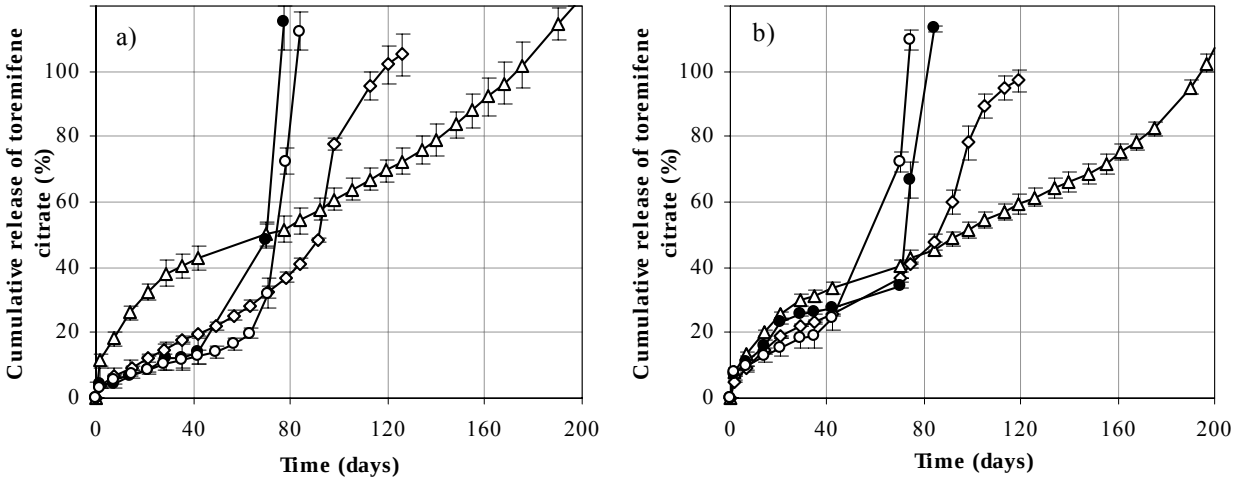


Figure 6. Cumulative release of toremifene citrate from a) copolymers and b) composites of these copolymers containing silica xerogel: P(CL80/LA20) (Δ), P(CL60/LA40) (◊), P(CL40/LA60) (○), P(CL20/LA80) (●). Results are averages (n=3) with standard error bars, (Data from I).

Results indicate that the release profiles of toremifene citrate were quite similar from both types of devices. The release profiles of toremifene citrate followed matrix-diffusion kinetics at the beginning, before a burst phase occurred. Silica xerogel delayed the beginning of the burst effect in the copolymer containing 20 wt.% ε-caprolactone but the desorption of the drug from the silica xerogel was not a significant factor in determining the rate or the profile of the release from any of the devices tested. Apparently, toremifene citrate was already partly released from the surface of silica xerogel during blending with the copolymer, and thus the effect of the silica xerogel on the release rate of the drug is not clear. Since P(CL80/LA20) provided the most uniform rate of toremifene citrate release, it was selected for drug loading studies with 2, 3, 4 and 10 wt.% drug in the copolymer. Figure 7 shows the cumulative release profiles for different drug loads. Increasing the initial drug loading increased the fraction of toremifene citrate released from the matrix, i.e. the relative release rate was constant and the amount released was found to be directly proportional to the toremifene citrate load when more than 2 wt.% was used. It can be speculated that 2 wt.% of the drug is dissolved in the matrix and thus the release rate observed is faster. The deviations from linearity with 10 wt.% load may be due to toremifene citrate catalysing the degradation of the matrix, leading to an increased release rate.

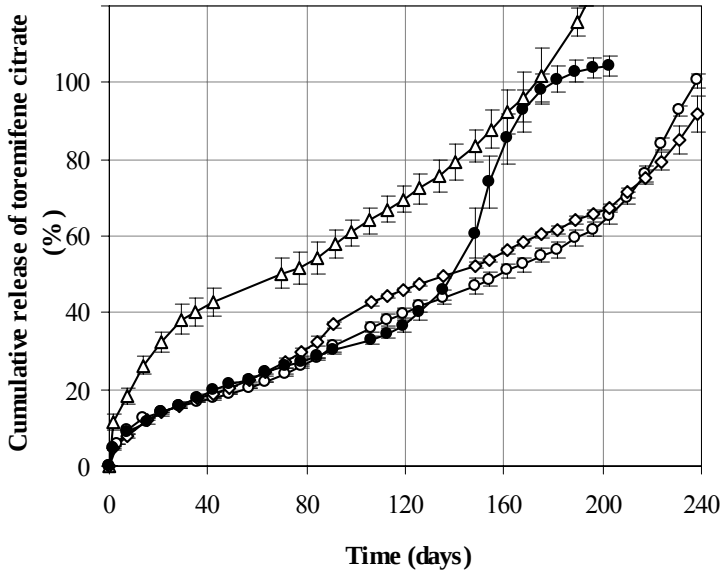


Figure 7. Effect of drug loading on the release from P(CL80/LA20) matrix, load 2 wt.% (Δ), 3 wt.% (◊), 4 wt.% (○), and 10 wt.% (●). Results are averages (n=3) with standard error bars, (Data from I).

3.1.2 Effect of the molecular weight on the release profile

The results of the first study indicated that the *in vitro* release of toremifene citrate could be adjusted by selecting an appropriate polymer composition and by varying the initial drug loading. The next point of interest involved the effects of the molecular weight of the copolymer and incorporation of the drug into silica xerogel during the sol gel process, instead of the adsorption method. Lower and higher molecular weight P(CL80/LA20) copolymers ($\overline{M}_w$ 60 000 g mol^{-1} (LMW) and $\overline{M}_w$ 300 000 g mol^{-1} (HMW)) were used in the devices. The level of the molecular weight was adjusted by the amount of glycerol co-initiator used in the ring-opening polymerisation. Copolymerisation of ε-caprolactone with 20 wt.% DL-lactide lowers the crystallinity compared with pure PCL, which should enhance the diffusion through the matrix. Also, the more hydrophilic lactide units promote the degradation of copolymer compared with PCL homopolymer. Matrix devices contained 2 wt.% toremifene citrate and composite devices contained 8.7 wt.% silica xerogel, corresponding to 2 wt.% drug content. Cumulative toremifene citrate releases from both types of device are shown in Figure 8.

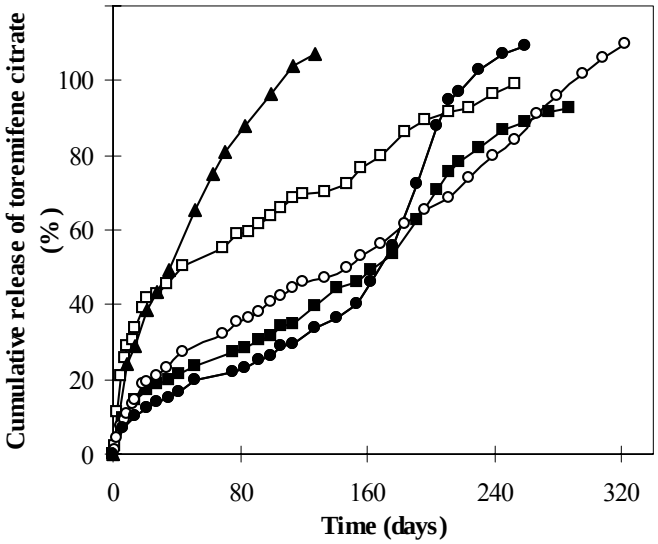


Figure 8. Release of toremifene citrate from the P(CL80/LA20) (copolymer/drug) device and the (copolymer /drug impregnated silica xerogel) composite devices. LMW device (○), LMW composite device (□), HMW device (●), HMW composite device (■), and HMW thin plate device (▲). Results are averages (n=3), error bars have been omitted for clarity (see Fig. 5 in II).

The release rate of toremifene citrate was steady for almost one year and no abrupt changes were observed in the release rate from LMW P(CL/LA) matrix and composite devices. Toremifene citrate release was clearly faster in the beginning from both matrix and composite devices made with LMW P(CL/LA) than from devices with HMW P(CL/LA). Similar findings regarding the role of the molecular weight of P(CL/LA) copolymer controlling the release rate have been reported by Wada *et al.* (1991). When the released amount (up to 70%) was plotted against the square root of the release time, a linear correlation was obtained (r=0.995). Thus, release rates determined from the slopes of the curves showed that toremifene citrate released at a constant rate of 4.54%/day^{½} from the LMW P(CL/LA)/drug matrix device according to square root of time kinetics. Toremifene citrate also released from the HMW P(CL/LA)/drug matrix devices according to square root of time kinetics (correlation r=0.948) at the rate of 3.5%/day^{½} before the abrupt change in the rate of release. The onset of faster drug release from HMW devices was after 160 days of hydrolysis, at which time the weight average molecular weight had decreased below 50 000 g mol^{-1}. It was clear that the degradation of the copolymer affected, and possibly preceded, the diffusion of the drug from the device. The incorporation of drug into silica xerogel moderated the change of release rate from HMW P(CL/LA) devices, but did not affect the release before the abrupt change in the rate of release. The release rate can be further modified by changing the device geometry (Wada *et al.*, 1991, Lemmouchi and Schacht, 1997), and thus it was confirmed that a good release profile and release duration of about three months was obtainable using a thin plate HMW P(CL/LA) device.

The degradation of polyesters proceeds in two stages. The first stage involves a decrease in molecular weight produced by non-enzymatic, random hydrolytic scission of ester cleavage,

and its duration is determined by the initial molecular weight of the polymer and its chemical structure (Pitt *et al.*, 1981b, Malin *et al.*, 1996). The second degradation stage is characterised by mass loss and a change in the rate of chain scission. The change in the rate of chain scission was observable for HMW P(CL/LA) after 182 days of hydrolysis (Figure 9). At that time, the mass loss was 10 wt.%. No change in the rate of chain scission was observed in the studied hydrolysis time for the LMW P(CL/LA), although the mass loss at 326 days of hydrolysis was 25 wt.%. The semilog plot of the *in vitro* rate of hydrolytic chain scission for the polymer samples over 300 days hydrolysis is shown in Figure 9.

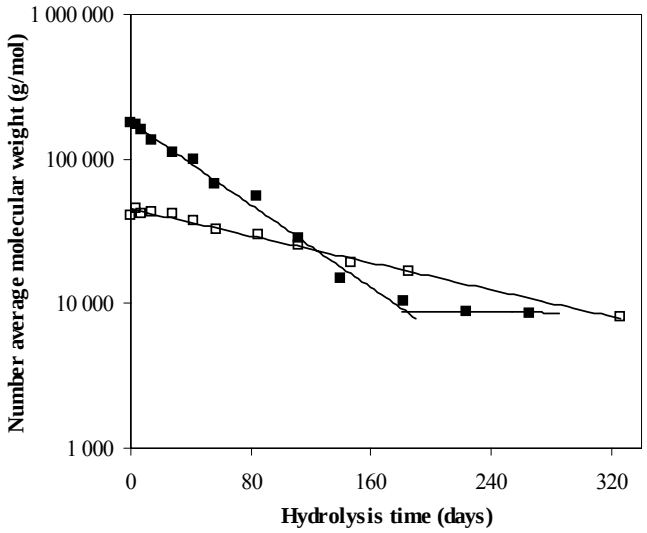


Figure 9. Semilog plot of the *in vitro* rate of hydrolytic chain scission of the P(CL80/LA20) copolymers LMW (□) and HMW (■). Results are averages (n=2) (II).

The weight average molecular weight of HMW P(CL/LA) samples containing drug or drug impregnated silica dropped 35% in just fourteen days of hydrolysis. On the other hand, the weight average molecular weight of LMW P(CL/LA) hardly changed at all over the same time. In general, the drug or silica impregnated samples degraded slightly faster than the polymer samples due to the higher water absorptions. This difference in degradation rate was more noticeable in HMW P(CL/LA) samples. The LMW copolymer structure may have been better protected against the hydrolysis, since the shorter chains can organise better and form crystallites more easily than the longer chains of HMW. The lactide content in HMW P(CL/LA) was slightly higher than in LMW P(CL/LA), which partly accounts for the faster hydrolysis rate. Based on the results in Publications I and II, it is feasible to formulate a device for further testing which is designed for long-term treatment of breast cancer after surgery.

3.2 Controlled release from poly(ε-caprolactone-co-DL-lactide)

The applicability of the P(CL/DL-LA) copolymers for matrix type controlled release devices was further studied in Publications III and IV. The aim of these studies was to investigate how the release rate from the copolymers can be modified by changing the copolymer composition, hydrophilicity of the copolymer and the amount of model compound in the device. In addition to Publications I and II, a more detailed characterization of the interactions between model drugs and copolymers was performed. Hydrolytic degradation of the copolymers was recorded, and thus the comparison between degradation and release profiles was obtained. The hydrophilicity of the copolymer matrix was altered using PEG co-initiators, since they have been found to increase the degradation rate and the hydrophilicity of the lactone polymers (Cohn and Younes, 1988, Kricheldorf and Meier-Haack, 1993, Huang *et al.*, 1997). The release of two model compounds, theophylline and propranolol hydrochloride, at different loadings (2-30 wt.%) was studied in Publication III, where the solubility of the model compounds was evaluated with DSC measurements. DL-lactide, instead of L-lactide, was again used as a comonomer, since amorphous polymers were preferred in the preparation of controlled release devices. In Publication IV, the release of theophylline, propranolol hydrochloride and lidocaine (10 wt.%), from copolymer with high lactide comonomer content was studied. It was of interest to see if the different model compounds would diffuse faster through the matrix compared to toremifene citrate studies and to compare the effects of hydrolytic degradation and release rates. Molecular modelling based on the molecular mechanics and dynamics simulations was performed in order to study the interactions between the theophylline model compound and homo- and copolymers of lactide and ε-caprolactone units. In the sample codes, the number after the code for theophylline (T), propranolol hydrochloride (P), or lidocaine (L) indicates the amount of model compound in the device as percentage weight.

3.2.1 Modification of copolymers by using PEG as a co-initiator

The properties of the different polymers used in studies III and IV are listed in Table 2. Total monomer conversion was nearly complete in the bulk polymerisation, since no monomer peaks could be observed in ^{1}H-NMR spectra. The hydrophilicity of the copolymers as well as the level of average molecular weight was modified using different co-initiators i.e. glycerol and polyethylene glycols. Contact angle measurements were carried out in order to establish differences in the hydrophilicity of the copolymers and lower contact angles were observed for polymers containing PEG. Caprolactone (10 wt.%) was introduced as a comonomer in P(DL-LA) in order to lower the glass transition temperature of amorphous homopolymer, which is around 55°C. As the temperature is lowered and approaches the glass transition temperature, the rate of diffusion decreases, reflecting a diminishing molecular motion (Plazek and Ngai, 1996). Copolymerisation lowered the glass transition temperature below body temperature to 30°C, and

this was expected to enhance the diffusion of the model compounds in the matrix. P(CL10/LA90) containing 90 wt.% of DL-lactide in the feed was amorphous and very hydrophilic due to the high lactide content. The molecular weight was moderate, which should also have enhanced diffusion compared to the high molecular weight polymer. The elastomeric copolymer matrix, P(CL60/LA40), was polymerised using 40 wt.% of DL-lactide in the feed.

Table 2. Characteristics of the (ε-caprolactone/DL-lactide) copolymers (Data from III and IV).

Polymer code	*Co-initiator and content*		*Monomer composition in feed CL/DL-LA*	*^{13}C NMR*		*SEC*			*DSC*	
				Average caproyl sequence length	*Average lactidyl sequence length*	$\overline{M}_n$	$\overline{M}_w$	*MWD*	T_g	T_m
(wt.%/wt.%)	*(mol%)*		*(mol%)*			*(g mol^{-1})*	*(g mol^{-1})*		*(°C)*	*(°C)*
P(CL95/LA5)	Glycerol	0.3	96/4	19.7	1.6	81 400	146 000	1.8	-59	52
P(CL/PEG4/LA)	PEG4000	0.1	96/4	17.3	1.9	67 800	155 000	2.3	-55	55
P(CL/PEG1/LA)	PEG1000	0.5	96/4	17.5	1.7	45 300	79 500	1.8	-55	52
P(CL60/LA40)	Glycerol	0.05	65/35	3.4	3.0	150 000	280 000	1.9	-31	-
P(CL10/LA90)	Glycerol	0.5	12/88	-[a)]	21.6	43 300	60 300	1.4	30	-

a) Not determined

3.2.2 Interactions of the model compounds with the copolymers

The solubility of the model compound into the polymer matrix affects the rate of release and thus it was of interest to estimate whether the model compounds were dissolved or dispersed into the polymer matrices. The release rate from dissolved monolithic systems is faster than the release rate from dispersed monolithic devices, i.e., according to Higuchi (1961), the higher the solubility the greater the release rate of the model compound.

Solubility of model compounds in P(CL/DL-LA) with minor lactide content

Introduction of the hydrophilic polyethylene glycol block did not alter the glass transition temperature of the modified copolymers (Table 3). The presence of model compounds, theophylline or propranolol hydrochloride, did not alter the T_g of copolymers either, and thus they are assumed to have low solubility in the copolymers. The changes in crystallinity of the samples were evaluated by comparing enthalpy of fusion values, which were calculated in theoretical proportions from DSC curves. The presence of small molecular weight compounds did not affect the crystallinity of the copolymers, except in P(CL95/LA5) T5, where the enthalpy increased by 9%. Flexible polyethylene glycol blocks in the copolymer backbone hinder the alignment of long macromolecular chains i.e. slightly lower crystallinity was observed in PEG modified copolymers.

In dispersed devices, the drug concentration exceeds the saturation solubility of drug in the polymer and discrete drug particles exist within the matrix. In such cases, the melting peak of the model compound can be determined by DSC. In order to study the solubility of theophylline (T_m 274.6°C) in the copolymer, the DSC was heated once up to 320°C. No theophylline peaks were seen in P(CL95/LA5) T2 and P(CL95/LA5) T5 samples. Melting of the theophylline crystals around 220°C was observed in all of the samples with higher loadings. The tendency of theophylline to exist in crystal form in a variety of samples suggests that it has a limited solubility in the copolymers used. The measured enthalpy values indicate that theophylline was soluble in copolymers when less than 10 wt.% was added, and was partly dispersed at higher loadings.

Table 3. Glass transition temperatures, melting temperatures and heat of fusion of P(CL/DL-LA) copolymers and theophylline model compound determined by DSC (Data from III).

Sample	*Polymer*			*Model Compound*	
	T_g *(°C)*	T_m *(°C)*	ΔH *(J/g)*	*(wt.%)*	ΔH *(J/g)*
Theophylline	-	-	-	100	117
P(CL95/LA5)	-56	52	66	0	-
P(CL95/LA5) T2	-55	52	64	2	-[a)]
P(CL95/LA5) T5	-55	53	72	5	-[a)]
P(CL95/LA5) T10	-55	54	63	10	70
P(CL95/LA5) T15	-56	53	64	15	120
P(CL95/LA5) T30	-55	54	62	30	117
P(CL/PEG1/LA)	-55	52	62	0	-
P(CL/PEG1/LA) T10	-55	53	53	10	50
P(CL/PEG4/LA)	-55	55	59	0	-
P(CL/PEG4/LA) T10	-54	56	60	10	60

a) no peak detected

All the samples containing propranolol hydrochloride showed a melting peak around 160°C (propranolol hydrochloride melts at 164.9°C), except P(CL95/LA5) P2. This indicates that only small amount of propranolol hydrochloride was dissolved in the matrix and the rest was dispersed. Propranolol hydrochloride had a lower solubility in P(CL/LA) copolymers compared to theophylline, since a larger portion of loaded propranolol chloride was found to exist in the crystal form.

Solubility of model compounds in amorphous copolymers

Of the three model compounds added to P(CL10/LA90), only lidocaine affected the glass transition temperature of the copolymer by shifting it down significantly, and thus acting as a plasticizer in the matrix. This, together with the fact that the polymer device remained transparent

after the introduction of lidocaine, indicates that it was dissolved in the matrix (10 wt.%). Scanning electron micrographs of the fracture surfaces of the copolymer with lidocaine and theophylline are shown in Figure 10.

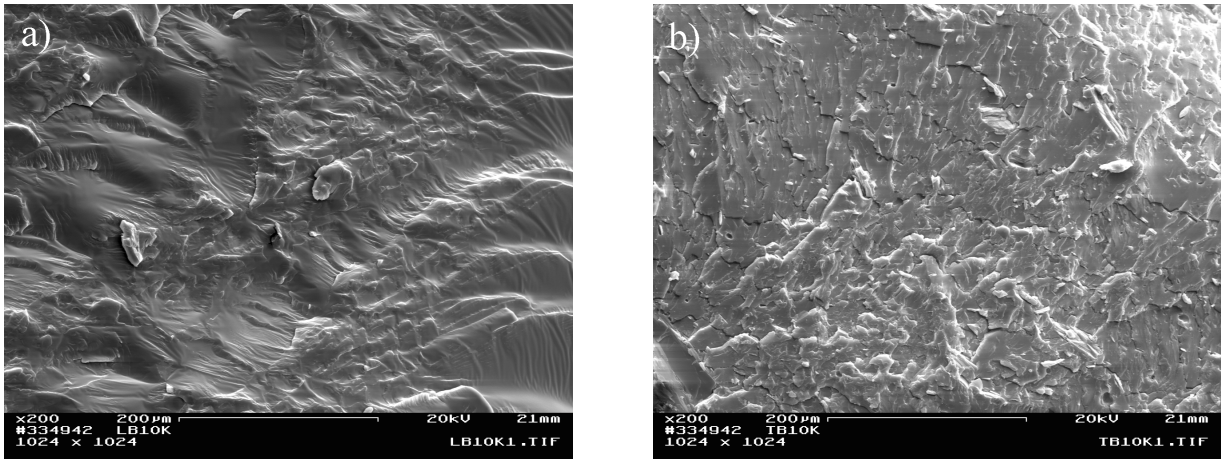


Figure 10. SEM micrographs of the P(CL10/LA90) copolymer containing 10 wt.% of a) lidocaine and b) theophylline (IV).

SEM micrographs revealed clear differences in the morphologies of the copolymer samples containing 10 wt.% of different model compounds. The morphology of the copolymer containing lidocaine appears smooth and no particles can be observed. On the other hand, the morphology of the samples containing theophylline or propranolol hydrochloride was rougher. A transparent device was also obtained when 10 wt.% theophylline was mixed in the P(CL60/LA40) matrix, although no significant change in the glass transition temperature was observed.

3.2.3 Estimating interactions by molecular modelling

In the Flexiblend method, the energy of mixing is estimated by calculating the interaction energies between short polymer chain segments (*A*) and a model molecule (*B*), as well as between like pairs *A*-*A* and *B*-*B*. The energy of mixing ΔE is then calculated:

$$\Delta E(\mathrm{AB}) = E_{\mathrm{AB}} - \frac{1}{2}(E_{\mathrm{AA}} + E_{\mathrm{BB}})$$

A negative result indicates that the compounds are miscible, i.e., the overall interactions between *A* and *B* are strong enough to provide miscibility. The more negative the result, the more likely it is that miscibility occurs.

Due to the local nature of interactions, only short chain segments need to be treated in the Flexiblend approach. For the calculations, three chain segments of approximately the same length were constructed: a chain of lactic acid units with five ester groups (PLA), a segment having a lactide unit (two lactic acid groups) between caprolactone units (P(CL-LA-CL)), and a chain

consisting of three ε-caprolactone units (PCL). In all cases, the segments ended with alkyl groups to avoid artificial effects due to the chain ends.

According to the AM1 calculations performed on theophylline, the molecule is almost planar. Additionally, theophylline is highly polar, the dipole moment being 6.5 D. The results from the Flexiblend calculations are presented in Table 4, which shows that for all polymer chain segments the mixing energies, and thus the interactions with theophylline, are small. Importantly, however, the calculations clearly show that increasing interaction between theophylline and the polymer matrix is obtained as the amount of lactide units increases.

Table 4. Results of the Flexiblend calculations (Data from IV).

Chain segment	ΔE_{tot} *(kcal mol^{-1})*
PLA	-0.9 ± 0.4
P(CL-LA-CL)	0.5 ± 0.4
PCL	0.7 ± 0.4

Figure 11 presents an optimised pair of theophylline and chain segment representing PLA obtained from the Flexiblend calculations. A detailed inspection shows that theophylline resides in a pocket held together by interactions between carbonyl oxygens in the ester groups of the polymer and carbonyl carbon in theophylline. Furthermore, carbonyl carbon in PLA interacts with the oxygen atom in the theophylline carbonyl group. These distances are all in the range of 2.9-3.1 Å. The planarity of theophylline enhances the possibility for simultaneous interactions from several directions, which makes the total effect of the interactions stronger.

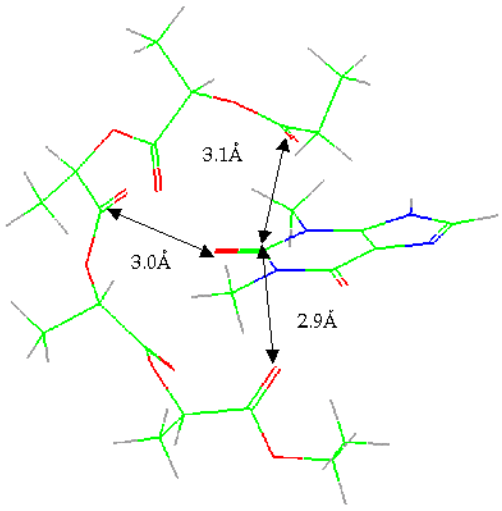


Figure 11. An example of an optimised pair of theophylline and PLA chain segment showing interactions between the functional groups in the molecules (IV).

3.2.4 Effect of comonomer ratio and hydrophilicity of the copolymer on the release profile

Cumulative release profiles of theophylline and propranolol hydrochloride (10 wt.%) from P(CL95/LA5) and PEG modified copolymers are shown Figure 12. The release profiles of different copolymer samples containing theophylline were similar. The initial release is fast from all copolymers. After approximately 30% is released, the release rate decreases and the differences between the copolymer matrices become evident. Faster theophylline release rates were obtained when the hydrophilicity of the copolymer was increased. An even greater effect on the release rate of propranolol hydrochloride was observed from PEG modification where the release flux of the hydrophilic drug was more than doubled from the modified matrix. The addition of hydrophilic polyethylene glycol blocks into the backbone of the chain increased water absorption and the degradation was faster when PEG 4000 was used as a co-initiator.

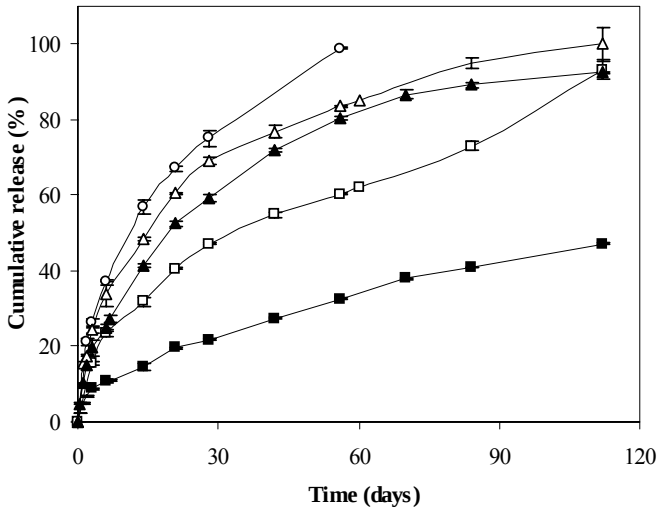


Figure 12. The cumulative release of theophylline (open symbols) and propranolol hydrochloride (closed symbols) from copolymers with different hydrophilicity. P(CL95/LA5) T10 (□), P(CL95/LA5) P10 (■), P(CL/PEG1/LA) T10 (Δ), P(CL/PEG1/LA) P10 (▲), P(CL/PEG4/LA) T10 (○). Results are averages (n=3) with standard error bars, (Data from III).

The addition of theophylline and propranolol hydrochloride at different loadings did not have an effect on the glass transition temperatures or melting temperatures of the P(CL95/LA5) copolymers, which indicates low interaction between the macromolecular chain and the model compounds. The solubility of the model compounds in the matrices was confirmed to be low by DSC measurements, where melting of the dispersed particles could be observed in most samples. Theophylline was soluble in copolymers when less than 10 wt.% was added and was partly dispersed at higher loadings. The difference in the release rate profiles was also evident when samples containing different amounts of theophylline, below and above the solubility limit, were compared. The release was initially faster from the samples where the model compounds were

molecularly dissolved (Figure 13). The release of both model compounds followed square root of time kinetics in dispersed devices.

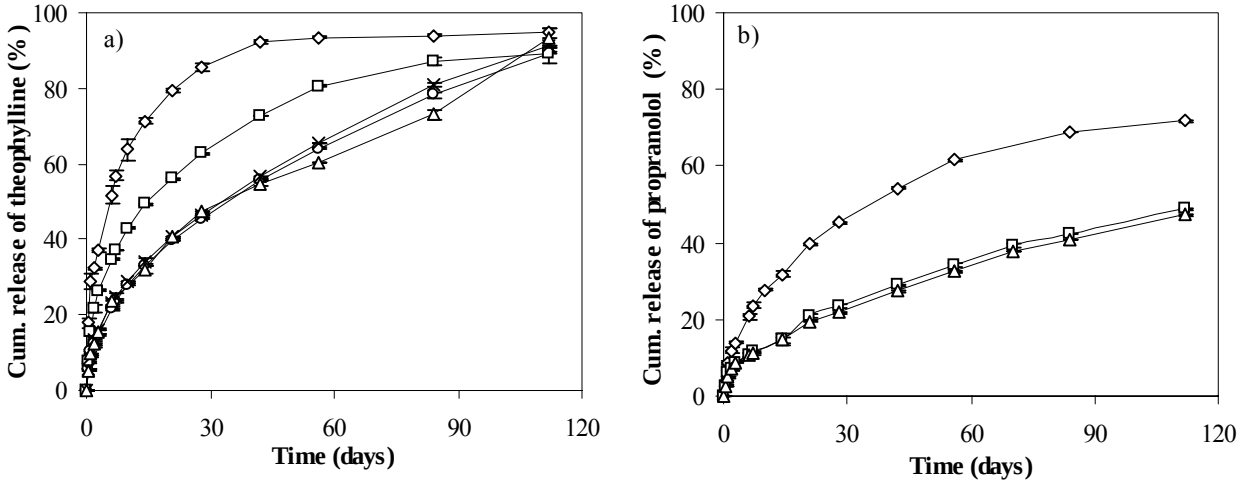


Figure 13. The effect of loading on the release rate of theophylline (a) and propranolol chloride (b) from P(CL95/LA5) copolymer. Drug load 2 wt.% (◊), 5 wt.% (□), 10 wt.% (Δ), 15 wt.% (○), and 30 wt.% (⁎). Results are averages (n=3) with standard error bars, (Data from III).

The release of three different model compounds from P(CL10/LA90) copolymer matrix as well as the change in the molecular weights of the copolymers is presented in Figure 14. In each case, release is ultimately governed by the matrix degradation. Diffusion controlled release is slow, and rapid release of the model compounds only occurs following a significant loss of molecular weight, after about 40 days of hydrolysis. At this time, the molecular weight of the copolymer was significantly reduced, the devices lost their shape, and a clear burst on the release profile was observed. There is a slight delay in the burst of propranolol hydrochloride but, in all cases, less than 20% of the release of the model compounds was diffusion controlled. The solubility of the model compound in the matrix material did not affect the release rate observed. Lidocaine was dissolved in the copolymer, but the release was nonetheless governed by the degradation of the matrix. The molecular modelling showed increasing interaction between theophylline and the polymer matrix as the amount of lactide units increased. This may partly explain the slow diffusion rate observed in the high lactide content copolymer matrix P(CL10/LA90).

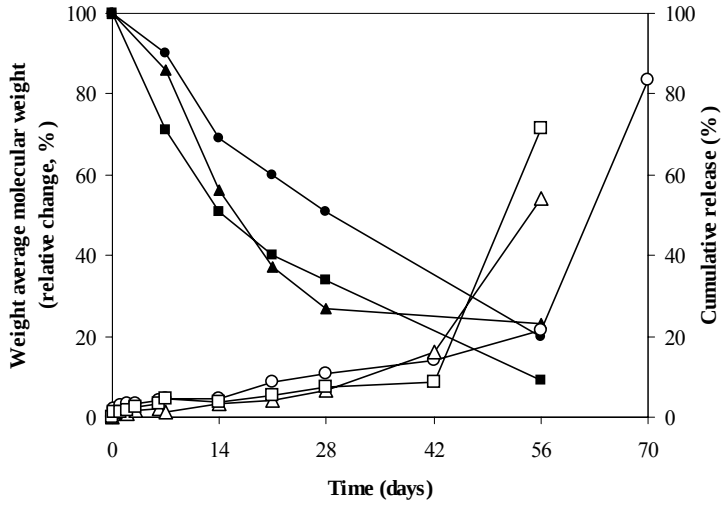


Figure 14. Cumulative release profiles of model compounds (10 wt.%, open symbols) from P(CL10/LA90) copolymer devices and their relative loss of molecular weight; (●) propranolol hydrochloride device, (■) lidocaine device, and (▲) theophylline device (IV).

The rate of degradation measured by the change in average molecular weight was not significantly affected by the presence of model compounds in any of the copolymers, which was rather surprising since there was a difference in the amounts of water absorbed. Water absorption over one month is 31% for theophylline, 55% for lidocaine, and 80% for propranolol hydrochloride containing P(CL10/LA90) devices, while the copolymer alone absorbs 30% water in 28 days. No vital change in the mass of the copolymers (besides model compound loss from devices) was observed in 28 days, although all devices lost their shape by that time and therefore water absorption and mass loss were not determined further.

Figure 15 shows the effect of the comonomer ratio on the release rates of theophylline. Constant, nearly zero order, release of theophylline was obtained with P(CL60/LA40) copolymer. Apparently, the amorphous nature of the copolymer together with caprolactone blocks enhanced the rate of the release compared with the copolymer of higher lactide content and semi-crystalline P(CL95/LA5) copolymer. Also, the higher solubility of theophylline compared to P(CL10/LA90) promoted the release. Due to its low T_g and lack of crystalline structure, the P(CL60/LA40) copolymer did not retain its form well at 37°C. Nevertheless, the copolymer provided a very steady rate of release and concurrent degradation of the device would enable repeated administration with a new device if required. The copolymer lost nearly 90% of its weight average molecular weight over the two months hydrolysis. The results clearly demonstrate that the desired release rates of these model compounds can be tailored by varying the compound loading, by modifying the hydrophilicity of the matrix copolymer from matrices with low lactide content and by choosing the appropriate comonomer ratio between ε-caprolactone and DL-lactide.

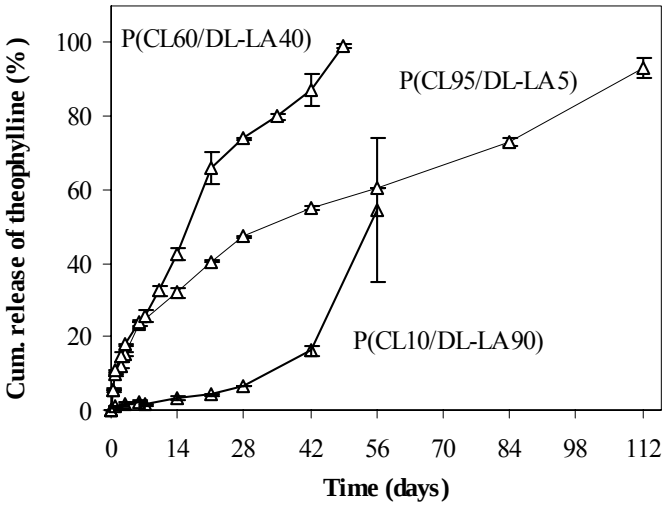


Figure 15. Release of 10 wt.% theophylline from P(CL/DL-LA) copolymers. Results are averages (n=3) with standard error bars, (Data from III and IV).

3.3 Bioresorbable composites for bone replacement

Composites of biodegradable polymers and bioactive glasses or glass-ceramics have been studied for a variety of applications in medicine. These include filling of bone defects (Higashi *et al.*, 1986, Ural *et al.*, 2000), guided tissue regeneration (Kellomäki *et al.*, 2000), fracture fixation (Shikinami and Okuno, 1999, Rokkanen *et al.*, 2000), tissue engineering (Hutmacher, 2000) and drug delivery as discussed here previously. Bioabsorbable devices are designed to maintain fixation during healing, then decompose gradually, transferring stress to the healing tissues. In this study, two different composite materials for bone replacement have been developed and their properties *in vitro* have been evaluated. The first material combined the well known characteristics of the P(CL/DL-LA) copolymer with bioactive glass S53P4 (Publication V) and the second was a composite consisting of amorphous lactic acid based PEU-BDI and bioceramic filler, HA or BCP (Publication VI).

3.3.1 Composite material of P(CL/LA) and bioactive glass S53P4 for use as a bone filler

The aim was to produce a composite material with combined beneficial properties for applications in orthopaedics and in oral and maxillofacial surgery. Lack of *in situ* mouldability, and the relative brittleness of bioactive glasses and glass ceramics limit the range of applications. Bioactive glasses and glass ceramics react both *in vivo* in body fluids and in several solutions *in vitro*. Due to ion dissolution, a silica gel layer is formed on the glass surface in the first hours, depending on the reactivity of the bioactive glass. This is followed by Ca-P precipitation and crystallisation resulting in a hydroxy apatite layer covering the surface. Silica gel formed on glass granules is considered to offer favourable nucleation sites for apatite formation (Ohtsuki *et al.*,

1992). Ideally, a composite material could combine the osteoconductive properties of bioactive glass with the processability of polymer to overcome limitations in the applicability of glass. Bioactive glass, S53P4, has shown good clinical performance in areas of compromised bone healing e.g. in the treatment of facial bone defects (Suominen and Kinnunen, 1996), obliteration of frontal sinuses (Peltola *et al.*, 1998) and maxillary sinus augmentation (Turunen *et al.*, 2002). A mouldable copolymer matrix enables the application and retention of the bioactive element at the operation site. The thermal properties of the P(CL/LA) copolymer can be adjusted to enable application by injection and moulding of the composite at relatively modest temperatures. Bioactive glass granules within the composite should be able to react and form an apatite layer on the composite surface, when exposed to body fluids. The biodegradation of the copolymer should also take place, enhancing the dissolution of bioactive glass granules and ultimately leading to bonding with the target tissue.

Different amounts of bioactive glass were incorporated in the P(CL95/DL-LA5) copolymer matrix in order to obtain slower (40 wt.%, equivalent to 23 vol.% glass) or accelerated bioactivity (60 or 70 wt.%, equivalent to 40 and 50 vol.% glass), i.e., slower or faster formation of a silica gel layer on the surface of the composite in dissolution. Two different granule size ranges of the bioactive glass were used, referred to as smaller (<45 μm) and larger (90-315 μm) range (Table 5). As the aim was to produce an injectable composite material, the molecular weight had to be such that the melt viscosity of the copolymer combined with the bioactive glass would not be too high. Use of the co-initiator (glycerol) allowed the molecular weight of the copolymer to be adjusted to the right level, i.e. weight average molecular weight of the copolymer was 110 000 g mol^{-1}. By choosing the right comonomer ratio of ε-caprolactone and DL-lactide, the melting temperature of the copolymer was lowered to 50°C.

Table 5. Composition of the bioactive composites consisting of P(CL/DL-LA) and bioactive glass used in study V.

Composite	*Bioactive glass*			
	granule size range	*in feed*		*measured*
	(μm)	*(vol.%)*	*(wt.%)*	*(wt.%)*
P(CL/DL-LA)	-	-	-	-
BG40S	<45	23	40	39.0±0.8
BG60S	<45	40	60	58.7±1.0
BG40L	90-315	23	40	39.9±0.3
BG60L	90-315	40	60	60.8±1.3
BG70L	90-315	50	70	71.0±1.9

The presence of filler changes the mechanical properties of the polymeric materials. Hardness and stiffness are usually increased, while impact and tensile strengths decrease. Filler,

which does not have strong interactions with the matrix material, increases the storage modulus both above and below the glass transition, but will not alter the position of the glass transition on the temperature axis (Gradin *et al.*, 1989). Bioactive glass does not interact with the P(CL/DL-LA) copolymer matrix, since the glass transition is not affected. The damping peak in DMTA measurements decreases with increasing glass content. Lowering of the damping energy suggests the restraining effect of the filler on the polymer segment mobility, demonstrating the increasing trend in composite rigidity.

The neat copolymer was fairly stable over six months hydrolysis due to the hydrophobic nature of the polycaprolactone blocks. Water absorption into the neat copolymer was less than one percent in the 168 days of hydrolysis. The presence of bioactive glass increased the water absorption, and the higher area/volume ratio of the smaller granule size range clearly enhanced the water absorption, up to 16%, compared to larger granules in the matrix even at 70 wt.% load. In composite materials, the fluid molecules diffuse into both matrix and filler phase. In this case, the majority of the diffusion occurs through the interface of the bioactive glass and the biodegradable polymer, since the polymer is relatively hydrophobic. The fluid diffuses through the interface by capillarity and microcrack transport mechanisms. In turn, the increased water uptake clearly had an effect on the rate of ester hydrolysis, since the average molecular weights of the composite samples were observed to decrease much faster. As random chain scission proceeds in the copolymer, smaller chain fragments are formed. The change in average chain length can be observed as an increasing difference between the number average and weight average molecular weight. Figure 16 shows the relative decrease of the average molecular weights for the polymer matrix and the composite containing 60 wt.% of bioactive glass of smaller range. The rate of chain scission is accelerated by the presence of the bioactive glass.

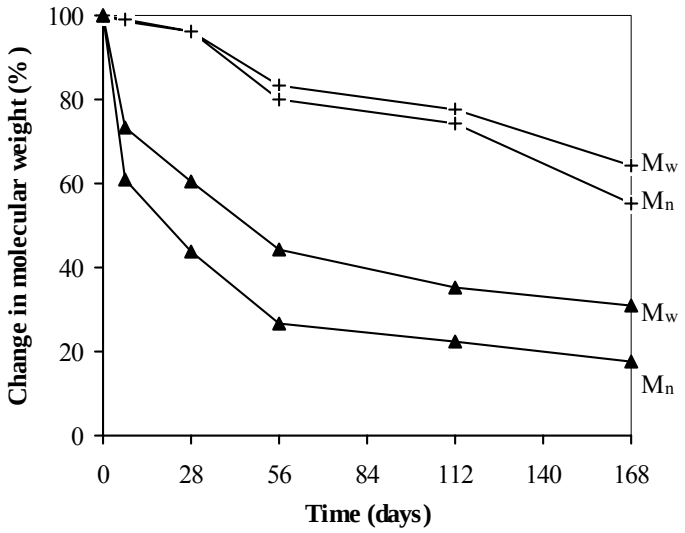


Figure 16. Changes in average molecular weights of P(CL/DL-LA) copolymer (+) and composite BG60S (▲) containing 60 wt.% of smaller range bioactive glass during hydrolysis in SBF at 37°C (Data from V).

Formation of Ca-P deposition on the surface of the composites after dissolution in SBF at 37°C was recorded by SEM. The degradation rate of the copolymer matrix has a detrimental effect on the bioactivity of the proposed injectable composite material. As formed, all the bioactive glass granules are intimately imbedded in the polymer matrix and even the near surface granules are covered by a polymer film of at least 1 μm thickness. Only the exposure of these granules, subsequent ion dissolution and formation of the Ca-P layer on the surface of the composite would enable bonding to soft tissue as well as hard tissue. Whereas the degradation rate of the P(CL/DL-LA) copolymer would not be fast enough to expose these granules, the combination of high glass content, increased water absorption and hence a faster degradation rate, provided enough enhancement so that differences in the surface properties of the different composites were evident after one week of hydrolysis. Higher water absorption and faster loss of molecular weight were observed in the composites containing the smaller granule size range of bioactive glass, namely <45 μm.

The first signs of Ca-P precipitation were observed on the composite containing 60 wt.% of the smaller glass after 24 hours of dissolution in SBF at 37°C, and within one week some deposition was observed on all of the composite materials. No surface precipitation was observed on the unfilled polymer sample. The fast deposition of Ca-P on the composite surface indicated fast ion dissolution and was probably due to the closer packing of the smaller bioactive glass granules with a higher surface/volume ratio in the matrix. Smaller granules could also pack better near the surface of the composite than larger granules with a lower surface/volume ratio. Ca-P crystals formed on the surface of the composite containing 60 wt.% of <45 μm glass after 3 weeks hydrolysis are shown in the SEM micrograph in Figure 17.

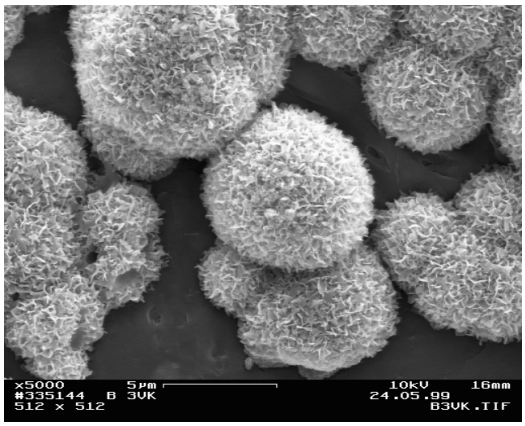


Figure 17. Formation of Ca-P deposition on the surface of the composite BG60S containing 60 wt.% of smaller bioactive glass after three weeks dissolution in SBF at 37°C (magnification x 5000) (V).

It was shown that a homogeneous distribution of the bioactive glass granules in the composites was obtained after compounding and compression moulding. Such uniform distribution is important in order to achieve the appropriate mechanical and biological performance of bioactive biodegradable composites. The formation of a biologically active Ca-P

layer on the surfaces of the composites in hydrolysis indicates *in vitro* bioactivity and it was found to be dependent on the weight fraction and granule size range of the bioactive glass used. The thermal properties of the polymer matrix would also enable the *in situ* application of the material. The presence of the bioactive glass affected the rate of the polymer degradation. The greater the amount and the smaller the granule size of the bioactive glass present, the more rapid was the deterioration in molecular weight of the samples. This can be attributed to the high water absorption in the composites accelerating the ester hydrolysis of the copolymer matrix. The correlation of *in vitro* and *in vivo* bioactivity of the composites needs to be established, but based on the *in vitro* evaluation, these composites have the potential for a variety of applications as implant materials in orthopaedics and dentistry.

3.3.2 Amorphous bone replacement material consisting of bioceramics and poly(ester-urethane)

As a matrix polymer, poly(lactic acids), are well known bioabsorbable and biocompatible polyesters, which are increasingly used as biomaterials for sutures, degradable films and fracture fixation. Here, an alternative route to obtain high molecular weight lactic acid based polymer was applied to obtain amorphous polymer matrix for bioactive fillers. This two-step synthesis route would enable further tailoring of the matrix, for example by varying the length of the prepolymer chains or comonomers. In this study, composites were prepared successfully by melt blending particulate bioceramic fillers, HA and BCP, at high, i.e. 20 and 40 vol.% loads (corresponding to 40 and 64 wt.%), with purified, thermoplastic lactic acid based PEU-BDI (M_w 175 000 g mol^{-1}). The aim was to develop a material that could withstand sterilization and retain the mechanical properties long enough for the healing to proceed. The effects of the γ-sterilization as well as the DMA properties in hydrolysis were characterized. DMA is useful to evaluate the viscoelastic properties of a polymer. The viscoelastic response is the direct reflection of the polymer structure, so it is commonly used to study the structure of a polymer and the interaction between the constituents of polymer composites.

The reinforcing effect of the fillers and filler content on the dynamic mechanical properties of the composites was clear, as shown in Figure 18. The stiffness of the materials, i.e. storage modulus values, increased with increasing filler content within the whole range of testing temperature, as is expected with the addition of rigid fillers. T_g values also increased (Table 6) as the filler content increased, indicating strong interactions between the filler and matrix. Widening of the glass transition region is due to the polymer becoming adsorbed onto the filler. Adsorption of polymer onto a surface restricts molecular motion, changes the density of packing of polymer chains and modifies the conformation and orientation of chain segments in the neighbourhood of the surface (Nielsen and Landel, 1994). Also strong secondary interactions between the ceramic fillers and poly(ester-urethane) can increase the T_g. The SEM micrographs of the cryogenic

fracture surfaces confirmed the strong interfacial bonding since no particle pullout was observed. Modulus values of the composites were within the ranges reported for cortical bone, 7-25 GPa.

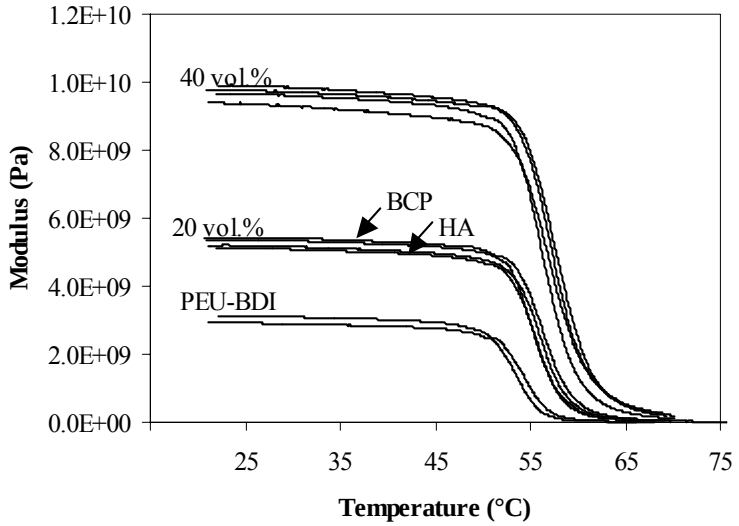


Figure 18. Storage modulus values of the composites and unfilled PEU-BDI before and after sterilization (VI).

The type of calcium phosphate filler did not affect the morphology of the composite; in all cases the fillers were homogeneously dispersed and in close contact with the polymer matrix. Average molecular weights and T_g values, determined from the peak of the loss modulus values, before and after γ-irradiation are shown in Table 6.

Table 6. Effects of gamma irradiation on molecular weights and glass transition temperatures of PEU-BDI and it's composites. DMTA measurements are averages of five with standard errors shown (VI).

Sample	*After processing*			*After radiation*		
	SEC		*DMTA*	*SEC*		*DMTA*
	$\overline{M}_w$ *(g mol^{-1})*	*MWD*	T_g *(°C)*	$\overline{M}_w$ *(g mol^{-1})*	*MWD*	T_g *(°C)*
PEU-BDI	170 000	2.3	54.2±0.2	102 000	2.2	53.5±0.2
PEU20HA	148 000	2.1	56.4±0.2	99 700	2.1	55.8±0.2
PEU40HA	123 000	2.2	57.6±0.5	86 500	2.1	56.4±0.6
PEU20BCP	153 000	2.4	56.3±0.3	99 300	2.2	55.0±0.5
PEU40BCP	137 000	2.4	57.3±0.2	99 300	2.2	57.3±0.2

The two major effects of radiation on a polymer are: chain scission occurring as a random rupturing of bonds, which reduces the molecular weight and the viscosity of the polymer, and cross-linking, which results ultimately in the formation of three-dimensional networks. Usually, both mechanisms occur simultaneously (Sintzel *et al.*, 1997). Composites were sterilized by a standard commercial γ-irradiation method using a nominal dose of 2.9 Mrad. Some random rupture of the polymer chains occurred, since the average molecular weights decreased by 30-

35%, as shown in Table 6. It is noteworthy that after sterilization all average molecular weights were similar, even though there were differences after processing. It seems that the polymer exhibits a threshold value of the molecular weight around 100 000 g mol^{-1}. This could be due to difference in the susceptibility of the different connecting groups in the polymer towards irradiation and thermal effects (Tuominen *et al.*, 2002). Higher doses of irradiation or more severe processing conditions would probably cause further destruction of polymer chains. Although some loss of molecular weight was observed, mechanical properties remained unchanged by irradiation and storage modulus values were similar to those before sterilization (Figure 18).

The hydrolysis of bulk degrading bioresorbable polymers usually proceeds by losing molecular weight at first, followed by loss of mass in the second stage when molecular weight has decreased to 15 000 g mol^{-1} or less (Pitt *et al.*, 1981b). In the hydrolysis of PEU-BDI and its composites, average molecular weights did not change much over five weeks, which was somewhat unexpected (Figure 19). When similar, but not purified poly(ester-urethane), which had been linked with 1,6-hexamethylene diisocyanate, was hydrolyzed at 37°C, the weight average molecular weight dropped by half during five weeks hydrolysis (Hiltunen *et al.*, 1998). In that case the water absorption was higher, at approximately 20%, but again no mass loss was observed. PEU-BDI is biodegradable, but the rate of degradation is significantly slower than is usually observed in amorphous poly(lactic acids). The biodegradation of PEU-BDI is clearly affected by the purification procedure, which is also known to affect the stability of PLAs (Hyon *et al.*, 1998). Solvent precipitation removes monomers or other short chains (e.g. dimer, lactide) as well as catalyst residuals, all of which can enhance the degradation rate in hydrolysis.

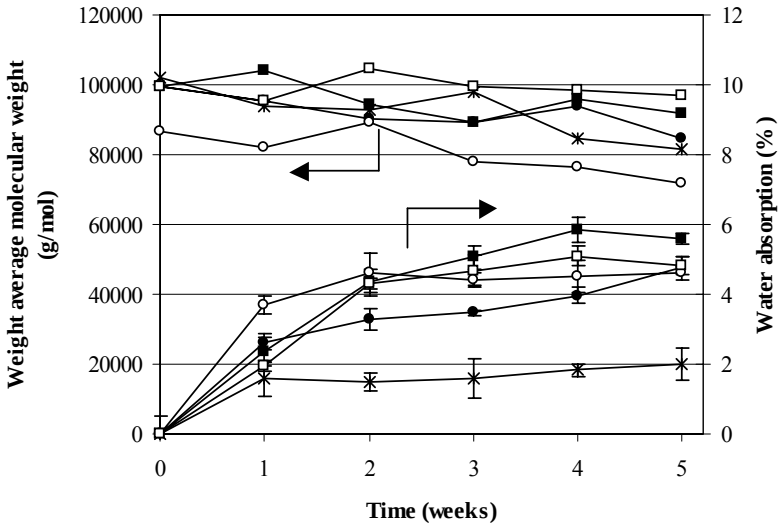


Figure 19. Water absorption and loss of molecular weight over five weeks of hydrolysis at 37°C; PEU-BDI (x), PEU20HA (●), PEU40HA (○), PEU20BCP (■), and PEU40BCP (□) (VI).

The amount or the type of filler did not influence the water absorption into the composite materials to a degree that would have affected the degradation of the polymer matrix (Figure 19).

The hydrolytic degradation of PEU-BDI was neither accelerated nor retarded by the presence of the fillers over the five weeks of hydrolysis. No mass loss was observed, since the degraded chains still remained long and showed no water solubility. Even though no major degradation occurred over 5 weeks, the dynamic mechanical analysis showed a decrease in the mechanical integrity of the composites during that time. The DMTA data showed different decreases in modulus values for the composites and PEU-BDI after immersion in saline. PEU-BDI retained its modulus well, whereas a faster loss of stiffness was observed in all the composite materials. This difference is attributed to the higher water absorption observed in the composites where the absorbed fluid concentrates at and disrupts the interface between the fillers and PEU-BDI matrix, easing deformation of the materials.

Relative modulus values, where the storage modulus of the composite is divided by the storage modulus of the unfilled polymer at the same temperature, were calculated at 37°C and are shown over the five-week hydrolysis period in Figure 20. Relative modulus values showed faster loss of rigidity at high filler content for HA containing samples than for BCP containing samples. The storage modulus values of all the composite materials remained within ranges that are mechanically compatible with bone over the whole five weeks of hydrolysis. Initially, the dynamic mechanical properties of the composites depended on the filler content and only minor differences between the composites containing either HA or biphasic calcium phosphate were observed. However, a faster loss of stiffness was observed in PEU40HA composites compared with PEU40BCP composite over the five-week *in vitro* hydrolysis. Relative modulus values are close for both fillers initially, but PEU40HA composites lose their stiffness faster than PEU40BCP composites. In general, the stiffness of the composite materials containing 40 vol.% ceramic filler reduced faster. The reinforcing effect of the fillers in the composite materials was retained over the hydrolysis time, since the relative modulus values remained above 1.0. It can be concluded that these materials have potential for application as fracture fixation materials.

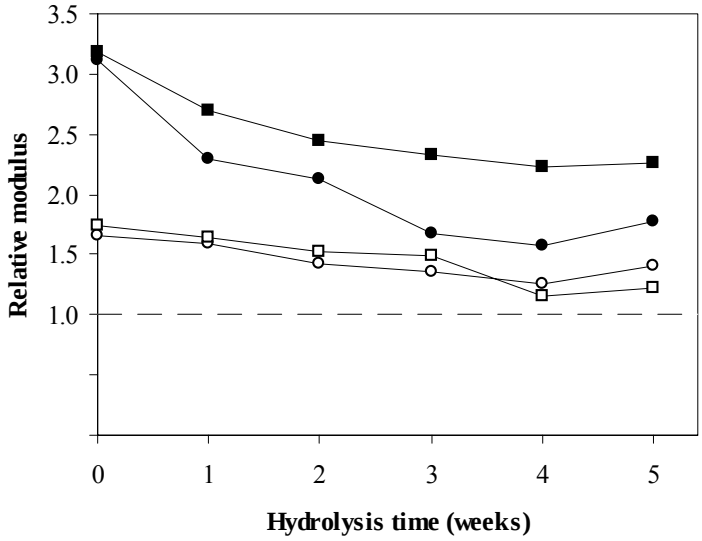


Figure 20. Change in the relative modulus of the composites PEU40BCP (■), PEU40HA (●), PEU20BCP (□) and PEU20HA (○) at 37°C over five weeks of hydrolysis (VI).

4 CONCLUSIONS

The bioresorbable polymers studied here were mainly synthesized by ring-opening polymerisation of ε-caprolactone and DL-lactide, but also by polycondensation of lactic acid followed by chain linking with BDI. The aim was to develop and on the other hand characterize polymer and composite materials that would be applicable as biomaterials and answer sufficiently the requirements for such materials from an engineering viewpoint. Polymers were tailored and characterized *in vitro* for different biomedical applications, aiming ultimately for controlled drug delivery or bone replacement devices. The following conclusions can be drawn from the studies presented in this thesis and in Publications I-VI:

- A biodegradable device for the controlled release of toremifene citrate was developed. The release period could be adjusted from 3 months to 1 year by varying the initial molecular weight of the poly(ε-caprolactone-co-DL-lactide), by incorporating toremifene citrate in silica xerogel in the composite device or by changing the dimensions of the device.

- The hydrophilicity of the poly(ε-caprolactone-co-DL-lactide) with minor lactide content was modified using different co-initiators, i.e. glycerol and poly(ethylene glycols). Increasing the hydrophilicity of the matrix also increased the release rates of model compounds used. The release of model compounds, theophylline and propranolol hydrochloride, followed square root of time kinetics in dispersed devices. Matrix devices consisting mostly of lactide were not found to be optimal for controlled release, since less than 20% of the release of the model compounds was diffusion controlled. Diffusion controlled release was slow, and the model compounds were only released rapidly following a significant loss of molecular weight. It was demonstrated that the desired release rates of the model compounds could be tailored by varying the compound loading, by modifying the hydrophilicity of the matrix copolymer and by choosing the appropriate comonomer ratio between ε-caprolactone and DL-lactide.

- Composite material for filling bone defects and use in guided tissue regeneration was developed by combining poly(ε-caprolactone-co-DL-lactide) copolymer with bioactive glass S53P4. The properties of the copolymer were adjusted to enable application by injection and moulding of the composite *in situ* at relatively modest temperatures. The formation of a biologically active Ca-P layer on the surfaces of the composites in hydrolysis indicated *in vitro* bioactivity and was found to be dependent on the weight fraction and granule size range of the bioactive glass used.

- A composite material consisting of amorphous lactic acid based poly(ester-urethane) reinforced with bioceramic fillers, HA or BCP, was developed for use as a bone replacement material. Melt blending and sterilization by gamma-irradiation caused some chemical degradation, but did not affect dynamic mechanical properties. The storage modulus values of all the composite materials remained within ranges that are mechanically compatible with bone over the whole five weeks of hydrolysis and are thought to have potential for application as fracture fixation materials.

ε-Caprolactone/DL-lactide copolymers are known to be biocompatible and their wide range of properties provides a good basis for their use in controlled drug release devices. The properties of the polymer/drug devices are always dependent on the specific combination and should thus be tested for desired profile. It is acknowledged that the effects of the sterilization should have been included in the study of these materials in all publications and that the biological and medical properties are yet to be established for these materials. However, the biocompatibility of reinforced poly(ester-urethane) composites has been assessed using an *in vitro* human osteosarcoma (HOS) cell culture model (Rich *et al.*, 2001). From the results of the cytotoxicity test, biochemical evaluation and morphological assessment, the polymer and composites were biocompatible with HOS cells and encouraged cell attachment and growth. Also, the evaluation of the biological behaviour of the bioactive glass composite materials has produced some promising results (Närhi *et al.*, 2002) and further development of the material for orthopaedic and dental applications is in progress.

REFERENCES

Ali, S. A. M., Zhong, S.-P., Doherty, P. J., Williams, D. F., 1993. Mechanisms of polymer degradation in implantable devices I Poly(caprolactone), *Biomaterials* 14, 648-656.

Amass, W., Amass, A., Tighe, B., 1998. A review of biodegradable polymers: Uses, current developments in the synthesis and characterization of biodegradable polyesters, blends of biodegradable polymers and recent advances in biodegradation studies, *Polym. Int.* 47, 89-144.

An, Y. H., Shane, K. W., Friedman, R. J., 2000. Pre-clinical *in vivo* evaluation of orthopaedic bioabsorbable devices, *Biomaterials* 21, 2635-2652.

Andersson, O. H., Liu, G., Karlsson, K. H., Niemi, L, Miettinen, J., Juhanoja, J., 1990. *In vivo* behaviour of glasses in the SiO_2-Na_2O-CaO-P_2O_5-Al_2O_3-B_2O_3 system, *J. Mater. Sci.: Mater. Med.* 1, 219-227.

Bajpai, P. K., Billotte, W. G., 1995. Ceramic Biomaterials. In *Biomedical Engineering Handbook*, Bronzino, J. D. (ed.), CRC Press, Florida, 552-573.

Baker, R., 1987. Diffusion controlled systems. In *Controlled Release of Biologically Active Agents*, John Wiley & Sons, New York, 39-83.

Barrows, T. H., 1991. Synthetic bioabsorbable polymers. In *High Performance Biomaterials*, Szycher, M. (ed.), Technomic Publishing Inc., Lancaster, 243-257.

Bergsma, J. E., Bruijn, W. C., Rozema, F. R., Bos, R. R. M., Boering, G., 1995. Late degradation tissue response to poly(L-lactide) bone plates and screws, *Biomaterials* 16, 25-31.

Berthiaume, F., Yarmush, M. L., 1995. Tissue Engineering. In *Biomedical Engineering Handbook*, Bronzino, J. D. (ed.), CRC Press, Florida, p. 1556.

Bleach, N. C., Nazhat, S. N., Tanner, K. E., Kellomäki, M., Törmälä, P., 2002. Effect of Filler Content on Mechanical and Dynamical Mechanical Properties of Particulate Biphasic Calcium Phosphate – Polylactide Composites, *Biomaterials* 23,1579-1585.

Bonfield W., Grynpas M. D., Tully A. E., Bowman J., Abraham J., 1981. Hydroxyapatite reinforced polyethylene: a mechanically compatible implant material for bone replacement, *Biomaterials* 2, 185-186.

Brink, M., Turunen, T., Happonen, R. P., Yli-Urpo, A., 1997. Compositional dependence of bioactivity of glasses in the system Na_2O-K_2O-MgO-CaO-B_2O_3-P_2O_5-SiO_2, *J. Biomed. Mater. Res.* 37, 114-121.

Buntner, B., Nowak, M., Bero, M., Dobrzynski, P., Kasperczyk, J., 1996. Controlled release of 17β-estradiol from D,L-lactide/ε-caprolactone copolymers, *J. Bioact. Comp. Polym.* 11, 110-121.

Chen, D., Chen, H., Bei, J., Wang, S., 2000. Morphology and biodegradation of microspheres of polyester-polyether block copolymer based on polycaprolactone/polylactide/poly(ethylene oxide), *Polym. Int.* 49, 269-276.

Cohn, D., Younes, H., 1988. Biodegradable PEO/PLA block copolymers, *J. Biomed. Mater. Res*. 22, 993-1009.

den Dunnen, W. F. A., Robinson, P. H., van Wessel, R., Pennings, A. J., van Leeuwen, M. B. M., 1997. Long-term evaluation of degradation and foreign-body reaction of subcutaneously implanted poly(DL-lactide-ε-caprolactone), *J. Biomed. Mater. Res.* 36, 337-346.

Domb, A. J., Bentolila, A., Teomin, D., 1998. Biopolymers as drug carriers and bioactive macromolecules,

Acta Polym. 49, 526-533.

Domb, A. J., Elmalak, O., Shastri, V. R., Ta-Shma, Z., Masters, D. M., Ringel, I., Teomim, D., Langer, R., 1997. Polyanhydrides. In *Handbook of biodegradable polymers*, Domb, A. J., Kost, J., Wiseman, D.M. (eds.), Harwood Academic Publishers, Amsterdam, 135-159.

Duda, A., Penczek, S, Kowalski, A., Libiszowski, J., 2000. Polymerizations of ε-caprolactone and L,L-dilactide initiated with stannous octoate and stannous butoxide – a comparison, *Macromol. Symp.* 153, 41-53.

Edgren, D., Leeper, H., Nichols, K., Wright, J., 1993. Controlled release technology (pharmaceutical). In *Kirk-Othmer*, 4th edition, vol 7, 274-300.

Gan, Z., Yu, D., Zhong, Z., Liang, Q., Jing, X., 1999. Enzymatic degradation of poly(ε-caprolactone)/poly(DL-lactide) blends in phosphate buffer solution, *Polymer* 40, 2859-2862.

Gogolewski, S., 2000. Bioresorbable polymers in trauma and bone surgery, *Injury, Int. J. Care Injured*, 31, S-D28-32.

Gradin, P., Howgate, P., Seldén, R., 1989. Dynamic-mechanical properties. In: Allen, G., Bevington, J. C. (eds.), *Comprehensive Polymer Science*. Pergamon Press, Oxford, U.K., p.533-569.

Griffith, L. G., 2000. Polymeric biomaterials, *Acta Mater.* 48, 263-277.

Grijpma, D. W., Zondervan, G. J., Pennings, A. J., 1991. High molecular weight copolymers of L-lactide and ε-caprolactone as biodegradable elastomeric implant materials, *Polymer Bulletin* 25, 327-333.

Han, D. K., Park, K. D., Hubbell, J. A., Kim, Y. H., 1998. Surface characteristics and biocompatibility of lactide-based poly(ethylene glycol) scaffolds for tissue engineering. In *Polymers for Tissue Engineering*, Scoichet, M. S., Hubbell, J. A. (Eds.), VSP BV, Netherlands, 221-234.

Heller, J., Barr, J., Ng, S. Y., Shen, H.-R., Schwach-Abdellaoui, K., Emmahl, S., Rothen-Weinhold, A., Gurny, R., 2000. Poly(ortho esters) – their development and some recent applications, *Eur. J. Pharm. Biopharm.* 50, 121-128.

Hench, L. L., Wilson, J., 1993. Introduction. In *An Introduction to Bioceramics*, Hench, L. L., Wilson, J. (eds.), World Scientific, Singapore, 1-24.

Hench, L. L., Splinter, R. J., Allen, W. C., Greenlee, T. K., 1971. Bonding mechanisms at the interface of ceramic prosthetic materials, *J. Biomed. Mater. Res. Symp.* 2, 117-141.

Hench, L. L., Andersson, Ö., 1993. Bioactive glasses. In *An Introduction to Bioceramics*, Hench, L. L., Wilson, J. (eds.), World Scientific, Singapore, 41-62.

Higashi, S., Yamamuro, T., Nakamura, T., Ikada, Y., Hyon, S.-H., Jamshidi, K., 1986. Polymer-hydroxyapatite composites for biodegradable bone fillers, *Biomaterials* 7, 183-187.

Higuchi, T., 1961. Rate of release of medicaments from ointment bases containing drugs in suspension, *J. Pharm. Sci.* 50, 874-875.

Hiljanen, M., 2001. Degradable lactic acid polymers modified by copolymerization and blending; Mechanical properties, *Acta Polytech. Scand. Chem. Technol. Ser.* 284, 1-47.

Hiljanen-Vainio, M., Kylmä, J., Hiltunen, K., Seppälä, J. V., 1997. Impact modification of lactic acid based poly(ester-urethanes) by blending, *J. Appl. Polym. Sci.* 63, 1335-1343.

Hiltunen, K., Seppälä, J. V., Härkönen, M., 1997. Lactic acid based poly(ester-urethanes): Use of hydroxyl terminated prepolymer in urethane synthesis, *J. Appl. Polym. Sci*. 63, 1091-1100.

Hiltunen, K., 1997. Synthesis and characterization of lactic acid based poly(ester-urethanes), *Acta Polytech. Scand. Chem. Technol. Ser.* 251, 1-56.

Hiltunen, K., Tuominen, J., Seppälä, J. V., 1998. The hydrolysis of lactic acid based ply(ester-urethane)s, *Polym. Int.* 47, 186-192.

Holy, C. H., Cheng, C., Davies, J. E., Shoichet, M. S., 2001. Optimizing the sterilization of PLGA scaffolds for use in tissue engineering, *Biomaterials* 22, 25-31.

Huang, Y., Chung, T., Tzeng, T, 1997. Drug release from PLA/PEG microparticulates, *Int. J. Pharm.* 156, 9-15.

Hutmacher, D. W., 2000. Scaffolds in tissue engineering bone and cartilage, *Biomaterials* 21, 2529-2543.

Hyon, S.-H., Jamshidi, K., Ikada, Y., 1998. Effects of residual monomer on the degradation of DL-lactide polymer, *Polym. Int.* 46, 196-202.

Ikada, Y., Tsuji, H., 2000. Biodegradable polyesters for medical and ecological applications, *Macromol. Rapid Commun.* 21, 117-132.

Jaakkola, T., Närhi, T., Hormia, M., Rich, J., Seppälä, J., Yli-Urpo, A., 2001. In vitro proliferation of the human fibroblasts on the P(ε-CL-co-D,L-LA)/bioactive glass composites, *Key Eng. Mater.* (Bioceramics 14) 218-220, 261-264.

Kallio, S., Kangas, L., Blanco, G., Johansson, R., Karjalainen, A., Perilä, M., Piippo, I., Sundqvist, H., Södervall, M., Toivola, R., 1986. A new triphenylethylene compound, Fc-1157a. I. Hormonal effects, *Cancer Chemother. Pharmacol.* 17, 103-108.

Kellomäki, M., Niiranen, H., Puumanen, K., Ashammakhi, N., Waris, T., Törmälä, P., 2000. Bioabsorbable scaffolds for guided bone regeneration and generation, *Biomaterials* 21, 2495-2505.

Klein, C. P. A. T., Dreissen, A. A., de Groot, K., 1983. Biodegradation behaviour of various calcium phosphate materials in bone tissue, *J. Biomed. Mat. Res.* 17, 769-784.

Kokubo, T., Kitsugi, T., Yamamuro, T., 1990a. Ca, P-rich layer formed on high-strength bioactive glass-ceramic A-W, *J. Biomed. Mater. Res.* 24, 331-343.

Kokubo, T., Kushitani, H., Sakka, S., Kitsugi, T., Yamamuro, T., 1990b. Solutions able to reproduce in vivo surface-structure changes in bioactive glass-ceramic A-W3, *J. Biomed. Mater. Res.*, 24, 721-734.

Korhonen H., Helminen A., Seppälä J. V., 2001. Synthesis of polylactide in the presence of co-initiators with different numbers of hydroxyl groups, *Polymer* 42, 7541-7549.

Kricheldorf, H., Meier-Haack, 1993. Polylactones. 22. ABA triblock copolymers of L-lactide and polyethylene glycol, *Makromol. Chem.* 194, 715-725.

Kylmä, J., Hiljanen-Vainio, M., Seppälä, J. V., 2000. Miscibility, morphology and mechanical properties of rubber modified biodegradable poly(ester-urethanes), *J. Appl. Polym. Sci*. 76, 1074-1084.

Kylmä, J., 2001. Lactic acid based poly(ester-urethane) – Modification via copolymerization, chain linking and blending, *Acta Polytech. Scand. Chem. Technol. Ser.* 282, 1-47.

Kylmä, J., Seppälä, J. V., 2001b. Ternary phase poly(ester-urethane)/elastomer/filler composites, *J. Appl.*

Polym. Sci. 79, 1531-1539.

Langer, R., 1995. Biomaterials and biomedical engineering, *Chem. Eng. Sci*., vol. 50, 4109-4121.

Lee, H. B., Kim, S. S., Khang, G., 1995. Polymeric biomaterials. In *Biomedical Engineering Handbook*, Bronzino, J. D. (ed.), CRC Press Inc., New York, 581-597.

LeGeros, R. Z., LeGeros, J. P., 1996. Calcium phosphate biomaterials in medical applications. In *Bioceram., Proc. Int. Symp. Ceram. Med.*, Kokubo T, Kakamura, T., Miyaji, F. (eds.), Elsevier Science, vol 9, 7-10.

Lemmouchi, Y., Schacht, E., 1997. Preparation and *in vitro* evaluation of biodegradable poly(ε-caprolactone-co-D,L-lactide)(X-Y) devices containing trypanocidal drugs, *J. Controlled Release* 45, 227-233.

Lemmouchi, Y., Schacht, E., Kageruka, P., De Deken, R., Diarra, B., Diall, O., Geerts, S., 1998. Biodegradable polyesters for controlled release of trypanocidal drugs: *In vitro* and *in vivo* studies, Biomaterials 19, 1827-1837.

Lewis, D. H., 1990. Controlled release of bioactive agents from lactide/glycolide polymers. In *Biodegradable Polymers as Drug Delivery Systems*, Chasin, M., Langer, R. (eds.), Marcel Dekker Inc., New York, 1-41.

Li, S., Girard, A., Garreau, H., Vert, M., 2001. Enzymatic degradation of polylactide stereocopolymers with predominant D-lactyl contents, *Polym. Degrad. Stab.* 71, 61-67.

Löfgren, A., Albertsson, A.-C., Dubois, P., Jérôme, R., 1995. Recent advances in ring-opening polymerization of lactones and related compounds, *J.M.S. – Rev. Macromol. Chem. Phys.* C35 379-418.

MacDonald, R. T., McCarthy, S. P., Gross, R. A., 1996. Enzymatic degradability of poly(lactide): Effects of chain stereochemistry and material crystallinity, *Macromolecules* 29, 7356-7361.

Malin, M., Hiljanen-Vainio, M., Karjalainen, T., Seppälä, J., 1996. Biodegradable lactone copolymers. II. Hydrolytic study of ε-caprolactone and lactide copolymers. *J. Appl. Polym. Sci*. 59, 1289-1298.

Martin, A, 1993. *Physical Pharmacy, Physical Chemical Principles in the Pharmaceutical Sciences*, 4. ed., Lea & Febiger, USA, p. 324-356.

Middleton, J. C., Tipton, A. J., 2000. Synthetic biodegradable polymers as orthopedic devices, *Biomaterials* 21, 2335-2346.

Miyajima, M., Koshika, A., Okada, J., Ikeda, M., Nishimura, K., 1997. Effect of polymer crystallinity on papaverine release from poly(L-lactic acid) matrix, *J. Controlled Release* 49, 207-215.

Miyajima, M., Koshika, A., Okada, J., Kusai, A., Ikeda, M., 1998. The effects of drug physico-chemical properties on release from copoly (lactic/glycolic acid) matrix, *Int. J. Pharm.* 169, 255-263.

Nielsen, L. E., Landel, R. F., 1994. Mechanical properties of polymers and composites. New York: Marcel Dekker, p. 131-217.

Närhi, T. O., Jansen, J. A., Jaakkola, T., de Ruijter, A., Ooms, E., Rich, J., Seppälä, J., Yli-Urpo, A., Bone response on degradable thermoplastic composite in rabbits, submitted to *Biomaterials* 2002.

Ohtsuki, C., Kokubo, T., Yamamuro, T., 1992. Mechanishm of apatite formation on CAO-SiO_2-P_2O_5 glasses in a simulated body fluid. *J. Non-Cryst. Solids* 143, 84-92.

Peltola, M., Suonpää, J., Aitasalo, K., Varpula, M., Yli-Urpo, A., Happonen, R. P., 1998. Obliteration of the frontal sinus cavity with bioactive glass, *Head Neck*, 20, 315-319.

Pitt, C. G., Chasalow, F. I., Hibionada, Y. M., Klimas, D. M., Schindler, A., 1981. Aliphatic polyesters. I. The degradation of poly(ε-caprolactone) *in vivo*, *J. Appl. Polym. Sci.* 26, 3779-3787.

Pitt, C. G., Gratzl, M. M., Kimmel, G. L., Surles, J., Schindler, A., 1981b. Aliphatic polyesters. II. The degradation of poly(DL-lactide), poly(ε-caprolactone), and their copolymers *in vivo*, *Biomaterials* 2, 215-220.

Pitt, C. G., Gratzl, M. M., Jeffcoat, A. R., Zweidinger, R., Schindler, A., 1979. Sustained drug delivery systems II: Factors affecting release rates from poly(ε-caprolactone) and related biodegradable polyesters, *J. Pharm. Sci.*, 68**,** 1534.

Pitt, C. G., 1990. Poly(ε-caprolactone) and its copolymers. In *Biodegradable Polymers as Drug Delivery Systems*, Chasin, M., Langer, R (eds.), Marcel Dekker Inc., New York, 71-120.

Plazek, D. J., Ngai, K. L., 1996. The glass transition temperature. In *Physical Properties of Polymers Handbook*, J.E. Mark, Ed., American Institute of Physics, p.139-159.

Ramakrishna, S., Mayer, J., Wintermantel, E., Leong, K., 2001. Biomedical applications of polymer-composites materials: a review, *Composites Science and Technology* 61, 118-1224.

Rich, J., Tuominen, J., Kylmä, J., Seppälä, J., Tanner, K. E., Di Silvio, L., *In vitro* biocompatibility of lactic acid based PEU/HA and PEU/BCP composites, proceedings of European Society for Biomaterials, London, September 12-14, 2001.

Rokkanen, P. U., Böstman, O., Hirvensalo, E., Mäkelä, E. A., Partio, E. K., Pätiälä, H., Vainionpää, S., Vihtonen, K., Törmälä, P., 2000. Bioabsorbable fixation in orthopaedic surgery and traumatology, *Biomaterials* 21, 2607-2613.

Schindler, A., Hibionada, Y. M., Pitt, C. G., 1982. Aliphatic polyesters. III. Molecular weight and molecular weight distribution in alcohol-initiated polymerizations of ε-caprolactone, *J. Polym. Sci.: Polym. Chem.* 20, 319-326.

Seal, B. L., Otero, T. C., Panitch, A., 2001. Polymer biomaterials for tissue and organ regeneration, *Mat. Sci. Eng. R*, 34, 147-230. Figure 1 is reprinted with permission from Elsevier Science.

Shikinami, Y., Okuno, M., 1999. Bioresorbable devices made of forged composites of hydroxyapatite (HA) particles and poly-L-lactide (PLLA): Part I. Basic characteristics, *Biomaterials* 20, 859-877.

Sintzel, M. B., Merkli, A., Tabatabay, C., Gurny, R., 1997. Influence of irradiation sterilization on polymers used as drug carriers - A review, *Drug Dev. Ind. Pharm.* 23, 857-878.

Suominen, E., Kinnunen, J., 1996. Bioactive glass granules and plates in the reconstruction of defects of the facial bones, *Scand. J. Plast. Reconstr. Hand. Surg.* 30, 281-289.

Thombre, A., Cardinal, J., 1988. Biopolymers for controlled drug delivery. In *Encyclopedia of Pharmaceutical Technology*, Swarbrick, J., Boylan, J.C., (Eds.), Marcel Dekker, New York , 61-88.

Tomihata, K., Suzuki, M., Oka, T., Ikada, Y., 1998. A new resorbable monofilament suture, *Polym. Degrad. Stab.* 59, 13-18.

Tuominen, J., Kylmä, J., Seppälä, J., 2002. Chain extending of lactic acid oligomers. 2. Increase of molecular weight with 1,6-hexamethylene diisocyanate and 2,2'-bis(2-oxazoline), *Polymer* 43, 3-10.

Turunen, T., Peltola, J., Yli-Urpo, A., Happonen, R.P., 2002. Bioactive glass granules as a bone substitute material in connection with maxillary sinus floor augmentation. *Clin. Oral. Impl. Res.* Submitted.

Törmälä, P., Pohjonen, T., 1997. Ultrahigh-strength self-reinforced polylactide composites and their surgical applications, *Macromol. Symp.* 123, 123-131.

Ural, E., Kesenci, K., Fambri, L., Migliaresi, C., Piskin, E., 2000. Poly(D,L-cactide/ε-caprolactone)/hydroxyapatite composites, *Biomaterials* 21, 2147-2154.

Valavaara, R., 1990. Toremifene, a triphenyl antiestrogen, in the treatment of breast cancer, Ph.D Thesis, University of Turku, Finland.

Vandamme, T., Mukendi, J.-F. N., 1996. Controlled release of levamisole from poly(ε-caprolactone) matrices I. Effects of the nature and the concentration of the drug incorporated into the matrices, *Eur. J. Pharm. Biopharm.* 43, 116-123.

Vert, M., 2000. Lactide polymerization faced with therapeutic application requirements, *Macromol. Symp.* 153, 333-342.

Vert, M., Li, S. M., Spenlehauerland, G., Guerin, P., 1992. Bioresorbability and biocompatibility of aliphatic polyesters, *J. Mater. Sci. Mater. Med*. 3, 432.

Wada, R., Hyon, S.-H., Nakamura, T., Ikada, Y., 1991. *In vitro* evaluation of sustained drug release from biodegradable elastomer. *Pharm. Res.* 8, 1292-1296.

Williams, D. F., 1990. Biocompatibility: Performance in the Surgical Reconstruction of Man, *Interdisciplinary Science Reviews* 15, 20-33.

Williams, D. F., 1987. *Definitions in Biomaterials*, Elsevier, Amsterdam, 71 p.

Williams, D. F., 1994. Biodeterioration/Biodegradation of polymeric medical devices *in situ*, *Int. Biodet. Biodeg.* 95-130.

Ye, W.-P., Chien, Y. W., 1996. Dual-controlled drug delivery across biodegradable copolymer. I. Delivery kinetics of levonorgestrel and estradiol through (caprolactone/lactide) block copolymer, *Pharm. Dev. Tech.* 1, 1-9.